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Katedra i Klinika Chorób Zakaźnych, Chorób Wątroby i Nabytych Niedoborów
Odpornościowych

Rozprawa doktorska

**OCENA ODPOWIEDZI HUMORALNEJ I DZIAŁAŃ NIEPOŻĄDANYCH
PO SZCZEPIENIU PRZECIW SARS-CoV-2**

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Wykaz skrótów

BMI – ang. body mass index, indeks masy ciała

COVID-19 – ang. corona-virus-disease-2019

CVST – ang. cerebral venous sinus thrombosis, zakrzepica zatok żylnych mózgu

EMA – ang. European Medicines Agency, Europejska Agencja Leków

IQR – ang. interquartile range, rozstęp ćwiartkowy

ITP – ang. immune thrombocytopenic purpura, pierwotna małopłytkowość immunologiczna

PEG – ang. polyethylene-glycol, glikol polietylenowy

RBD -ang. receptor binding domain, domena wiążąca receptor

SARS-CoV-2 – ang. severe acute respiratory syndrome coronavirus 2, zespół ciężkiej ostrej niewydolności oddechowej koronawirus 2

WHO – ang. World Health Organization, Światowa Organizacja Zdrowia

VE – ang. vaccine efficacy, skuteczność szczepienia

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I. Nota informacyjna – wykaz publikacji wchodzących w skład cyklu

Rozprawa doktorska powstała w oparciu o cykl 3 prac oryginalnych.

Badania będące podstawą poniższych prac zrealizowano po uzyskaniu pozytywnej opinii Komisji Bioetycznej przy Uniwersytecie Medycznym we Wrocławiu (uchwały nr: KB - 51/2021 z dnia 29 stycznia 2021 r. i KB - 379/2021 z dnia 26 listopada 2021 r.). Każdy z artykułów został opublikowany w anglojęzycznych czasopismach naukowych o zasięgu międzynarodowym. Łączna wartość współczynnika wpływu impact factor (IF) zbioru prac wynosi 10,2, a liczba punktów Ministerstwa Edukacji i Nauki (MEiN) 320.

Publikacje wchodzące w skład zbioru:

- 1) **Monika Stepień**, Natalia Świątoniowska-Lonc, Brygida Knysz, Beata Jankowska-Polańska, Amadeusz Kuźniarski, Agnieszka Piwovar, Małgorzata Zalewska; 2023; *'An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland'*; Advances in Clinical and Experimental Medicine; IF 2,1; punkty MEiN 140
- 2) **Monika Stepień**, Małgorzata Zalewska, Brygida Knysz, Natalia Świątoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łaczmański, Agnieszka Piwovar, Amadeusz Kuźniarski; 2022; *'How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection'*; Vaccines; IF 7,8; punkty MEiN 140
- 3) **Monika Stepień**, Małgorzata Zalewska, Amadeusz Kuźniarski, Beata Jankowska-Polańska, Agnieszka Piwovar, Natalia Świątoniowska-Lonc, Brygida Knysz; 2023; *'How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2'*; Advances in Hygiene and Experimental Medicine; IF 0,3; punkty MEiN 40

Oświadczenia współautorów prac, określające wkład każdego z nich, zawarte są w załącznikach w części XII a.

II. Streszczenie

Wstęp:

Pierwsze informacje dotyczące ciężkiego zapalenia płuc wywołanego przez wirusa SARS-CoV-2 pojawiły się pod koniec 2019 r., a w grudniu 2020 r. pierwsze szczepionki przeciw SARS-CoV-2 zostały wprowadzone do powszechnego użytku. W Polsce zarejestrowanych jest pięć szczepionek: dwie szczepionki mRNA – BNT162b2 (Comirnaty; Pfizer-BioNTech) i mRNA-1273 (Spikevax; Moderna), dwie szczepionki wektorowe – ChAdOx1 nCoV-19 (Vaxzevria; Oxford-Astra Zeneca) i Ad26.COV2.S (Jcovden; Janssen-Johnson & Johnson) oraz szczepionka białkowa NVX-CoV2373 (Nuvaxovid; Novavax). Infekcja SARS-CoV-2 i/lub szczepienie skutkowały immunologiczną odpowiedzią humoralną i powstaniem przeciwciał neutralizujących specyficznych dla SARS-CoV-2. Wraz z powszechnym dostępem do szczepionek, rozpoczęły się liczne badania dotyczące ich skuteczności i bezpieczeństwa.

Cele:

1. Ocena poziomu przeciwciał anty-SARS-CoV-2 IgG u ozdowieńców i osób wcześniej niezakażonych (naiwnych), planujących zaszczepienie się przeciw SARS-CoV-2.
2. Pomiar stężeń przeciwciał po pierwszej dawce szczepienia, po drugiej dawce/po pełnym cyklu szczepienia (w przypadku jednodawkowych schematów przewidzianych dla preparatu Ad26.COV2.S) oraz porównanie ich z wyjściowymi poziomami przeciwciał. Analiza najczęściej występujących poszczepiennych działań niepożądanych, w zależności od zastosowanego preparatu, jego dawki oraz ewentualnej infekcji SARS-CoV-2 w przeszłości.
3. Ponowna ocena poziomu przeciwciał 6 miesięcy po zakończeniu schematu podstawowego szczepienia, z uwzględnieniem skuteczności szczepień (wystąpieniem zachorowań).

Materiał i metody:

Wszystkie trzy prace dotyczą pacjentów, którzy zgłosili się do badania przed przyjęciem szczepienia przeciwko SARS-CoV-2. Pierwotnie zostało zakwalifikowanych 298 osób, 171 ozdowieńców (grupa I) i 127 naiwnych pacjentów (grupa II). Zarejestrowani zgłaszali się w

wyznaczonym terminie na pobranie pierwszej próbki krwi (D0) do oceny poziomu przeciwciał. Na kolejne pobranie krwi pacjenci zgłaszali się po pierwszej dawce szczepienia (D1 = 286 osób: grupa I – 163, grupa II – 123) oraz po drugiej dawce (D2 = 295 osób: I - 169, II - 126). Zdecydowana większość przyjęła szczepionki mRNA (BNT162b2 n=191, mRNA-1273 n= 70). Porównując wyniki z etapów D1 i D2, u części pacjentów zaobserwowano kontrintuicyjny spadek stężenia przeciwciał po otrzymaniu drugiej dawki. Choć zjawisko to wystąpiło niezależnie od rodzaju przyjętego preparatu, w celu ograniczenia czynników mogących wpływać na wyniki, do kontrolnych badań 6 miesięcy po szczepieniu zrekrutowano 87 osób, które otrzymały 2 dawki BNT162b2.

Wyniki:

Po wykonaniu pierwszych badań, wśród ozdowieńców (grupy I) zdołano wyodrębnić 3 podgrupy: podgrupa I – pacjenci seropozytywni, którzy przebyli zakażenie SARS-CoV-2 asymptomatycznie (n = 21, 12,28 % grupy I i 7,05% całej grupy badanej), podgrupa II – ozdowieńcy objawowi seronegatywni (n = 23, 13,45% grupy I i 7,72% całej grupy), podgrupa III – ozdowieńcy z potwierdzoną infekcją SARS-CoV-2 w przeszłości, seropozytywni (n = 127). W podgrupie III wiek był istotnie wyższy niż w podgrupie II, podobną zależność można zaobserwować w porównaniu podgrupy III z grupą II (pacjentów naiwnych). 100% uczestników odpowiedziało na szczepienie po zakończeniu schematu podstawowego. Po pierwszej dawce u grupy I zaobserwowano 52-krotny wzrost miana przeciwciał. Dla grupy II, po drugiej dawce, zaobserwowano prawie 5-krotny wzrost. Immunologiczna stymulacja grupy I po pierwszej dawce była silniejsza niż grupy II po drugiej dawce (mediana 4736,0 vs. 3056,0 IU/ml; p = 0,00003). U 61 ozdowieńców (37,7% grupy I) poziomy przeciwciał po drugiej dawce okazały się niższe niż przed jej przyjęciem, co mogło być związane ze szczególnie wysokimi stężeniami przeciwciał po pierwszej dawce (p = 0,0001), możliwie także z młodszym wiekiem (p = 0,0418). Po 6 miesiącach od szczepienia BNT162b2, wśród 87 przebadanych osób, żadna nie przebyła objawowej infekcji. Różnice między ozdowieńcami ze stałym wzrostem poziomu przeciwciał (A), a tymi z odnotowanym spadkiem (C) zostały niemalże zatarte (p = 0,048).

Wnioski:

- Szczepienie przeciw COVID-19, niezależnie od rodzaju zastosowanej szczepionki, u wszystkich pacjentów było skuteczne i indukowało odpowiedź humoralną. Objawy niepożądane, które wystąpiły u części osób szczepionych, nasilające się po kolejnej dawce antygeny (wirus lub szczepionka), nie miały istotnego wpływu na stan zdrowia
- Objawy COVID-19 były częściej obserwowane u starszych pacjentów, a ciężkość objawów może być powiązana z wyższymi stężeniami przeciwciał anti-SARS-CoV-2
- Część pacjentów może nie prezentować objawów infekcji SARS-CoV-2, pozostając przy tym źródłem zakażenia dla zdrowych osób z otoczenia
- Obecność lub brak przeciwciał anti-SARS-CoV-2 IgG nie może być jedynym czynnikiem determinującym stwierdzenie zakażenia SARS-CoV-2 w przeszłości
- Wszystkie cztery rodzaje szczepień spowodowały odpowiedź humoralną zarówno u ozdowieńców jak i pacjentów naiwnych, ze szczególnym wskazaniem na preparaty mRNA (mRNA-1273 oraz BNT162b2)
- U ozdowieńców po przyjęciu pierwszej dawki zaobserwowano istotnie wyższe poziomy przeciwciał niż u osób naiwnych po drugiej dawce, co świadczy o silniejszym pobudzeniu immunologicznym
- Wśród ozdowieńców zaszczepionych preparatami mRNA-1273, BNT162b2 i ChAdOx1 nCoV-19, 37,7% wykazało spadek poziomu przeciwciał po drugiej dawce w porównaniu do wyników sprzed jej przyjęcia, co może wskazywać na zasadność wydłużania interwału czasowego między kolejnymi dawkami u ozdowieńców
- W grupie pacjentów naiwnych objawy niepożądane były silniej wyrażone po przyjęciu drugiej dawki szczepienia, podczas gdy u ozdowieńców były często obserwowane już po pierwszej dawce
- Niezależnie od stężenia przeciwciał uzyskanych na wcześniejszych etapach badania, żaden pacjent nie manifestował objawów infekcji SARS-CoV-2 w ciągu 6 miesięcy od przyjęcia drugiej dawki szczepienia
- Czasowy spadek poziomu przeciwciał u części ozdowieńców po drugiej dawce nie miał istotnego wpływu na wyniki badań po 6 miesiącach

III. Summary

Background:

The first information about severe respiratory syndrome caused by coronavirus 2 (SARS-CoV-2) emerged at the end of 2019 and at the end of 2020 the first vaccinations against SARS-CoV-2 were introduced into general use. In Poland five types of vaccines are available: two mRNA vaccines - BNT162b2 (Comirnaty; Pfizer-BioNTech) and mRNA-1273 (Spikevax; Moderna), two vector vaccines ChAdOx1 nCoV-19 (Vaxzevria; Oxford-Astra Zeneca) and Ad26.COV2.S (Jcovden; Janssen-Johnson & Johnson) and protein-based vaccine NVX-CoV2373 (Nuvaxovid; Novavax). SARS-CoV-2 infection and/or vaccination resulted in immunological humoral response and production of neutralizing antibodies specific for SARS-CoV-2. Along with universal access to the vaccines, numerous studies regarding their safety and efficacy started.

Objectives:

1. Assessment of anti-SARS-CoV-2 IgG antibodies in convalescents and people never infected (naive), planning to get vaccinated.
2. Measurement of the antibody concentration after first dose, second dose/ full vaccine schedule (one dose for Ad26.COV2.S) and comparison of the results with baseline antibody levels. Analysis of the most common adverse effects after the vaccinations, depending on the vaccine type, dose and possible infection in the past.
3. Reassessment of antibody levels 6 months after basic vaccination schedule, with efficacy control (occurrence of disease).

Material and methods:

All three manuscripts concern patients, who volunteered for the study before receiving the vaccine against SARS-CoV-2. Primarily 298 people were qualified, 171 convalescents (group I) and 127 naive patients (group II). Registered people came on the scheduled date for first blood test (D0) to check antibody levels. For the next blood cells patients showed up after the first dose (D1 = 286 people: group I – 163, group II – 123) and after second dose (D2 = 295

people: I - 169, II - 126). The vast majority got mRNA vaccines (BNT162b2 n=191, mRNA-1273 n= 70). While comparing the results from stages D1 and D2, in some convalescents a counterintuitive decline of antibody levels was observed after the second dose. Although the phenomenon did not depend on the type of the vaccines, in order to limit the factor which may influence the results, for the reassessment 6 months after the vaccination 87 people vaccinated only with BNT162b2 were invited.

Results:

After first blood testing, among the convalescents (group I) 3 subgroups were found: subgroup I – patients seropositive, who were infected with SARS-CoV-2 asymptotically (n = 21, 12,28 % of group I and 7,05% of the whole study group), subgroup II – convalescents symptomatic, seronegative (n = 23, 13,45% of group I and 7,72% of the whole study group), subgroup III – convalescents with confirmed SARS-CoV-2 infection in the past, seropositive (n = 127). In subgroup III age was significantly higher than in subgroup II, similar relationship may be observed between subgroup III and group II (naïve patients). 100% of the participants responded to the vaccinations after full schedule. After first dose in group I a 52-fold increase of antibody levels was observed. In group II, almost 5-fold increase was observed. Immunological stimulation of group I after first dose was stronger than group II after second dose (median 4736,0 vs. 3056,0 IU/ml; $p = 0,00003$). In 61 convalescents (37,7% of group I) the antibody levels after the second dose turned out to be lower than before receiving it, which could be connected with especially high antibody titers after the first dose ($p = 0,0001$), possibly also with younger age ($p = 0,0418$). 6 months after BNT162b2 vaccination, among 87 tested participants, none underwent a symptomatic infection. The differences between convalescents with constant increase of antibody levels (A) and those with reported decrease (C) were almost blurred ($p = 0,048$).

Conclusions:

- Vaccinations against COVID-19, independently from the type of used vaccine, were effective in all patients and induced humoral response. The adverse effects present in

some vaccinated patients, stronger after next dose of antigen (virus or vaccine), did not have an important impact on health condition

- COVID-19 symptoms were more often observed in older participants and the severity of the disease can correlate with higher antibody titers
- Some patients may not present symptoms, yet they may be the source of the infection for other healthy people
- Serological data are not an unambiguous evidence of a past infection
- All four types of vaccines induced serological responses in both convalescents and naive patients, particularly mRNA specimens (mRNA-1273 and BNT162b2)
- In convalescents after receiving first dose significantly higher antibody levels were observed in comparison with naive patients after second dose, which proves a stronger immunological stimulation
- Among convalescents vaccinated with mRNA-1273, BNT162b2 and ChAdOx1 nCoV-19 specimens, 37,7% presented a decrease of antibody levels after the second dose in comparison with the results before receiving it, which may indicate a necessity to extend the time intervals between next vaccine doses for convalescents
- In naive group, adverse events were more intensified after second dose of the vaccine, whereas in the convalescents, they were often observed after the first dose
- Independently of antibody levels on previous stages of the study, none of the patients suffered from symptomatic SARS-CoV-2 infection within 6 months after vaccination
- Temporary decrease of antibody levels in convalescents after the second dose of SARS-CoV-2 vaccination had no important influence on antibody level results 6 months after vaccination.

IV. Wstęp

Pierwsze informacje dotyczące ciężkiego zapalenia płuc wywołanego przez wirusa SARS-CoV-2 pojawiły się pod koniec 2019 r. Szybkie rozprzestrzenienie się nowego patogenu doprowadziło do wybuchu pandemii, ogłoszonej 11 marca 2020 r. przez WHO [1]. W krótkim czasie świat doświadczył obostrzeń na niespotykaną dotychczas skalę. Miliony ludzi ucierpiało nie tylko z powodu objawowego zakażenia i medycznych powikłań zachorowania na COVID-19 wywołanego przez SARS-CoV-2, ale także w wyniku ograniczeń i wymuszonych zmian epidemiologiczną zmian w codziennym życiu prywatnym i zawodowym. Naturalną odpowiedzią środowiska medycznego na zaistniałą sytuację było zaangażowanie niezliczonej ilości naukowców w poszukiwanie skutecznych leków na COVID-19 oraz szczepionek umożliwiających ochronę przed zakażeniem i jego najgroźniejszymi skutkami. W grudniu 2020 r. pierwsze szczepionki przeciw SARS-CoV-2 zostały wprowadzone do powszechnego obiegu. W Polsce zarejestrowanych jest pięć szczepionek, które EMA dopuściła do użytku. Dwie z nich to szczepionki mRNA – BNT162b2 (Comirnaty; Pfizer-BioNTech) i mRNA-1273 (Spikevax; Moderna), dwie szczepionki wektorowe – ChAdOx1 nCoV-19 (Vaxzevria; Oxford-Astra Zeneca) i Ad26.COV2.S (Jcovden; Janssen-Johnson & Johnson) oraz szczepionka białkowa NVX-CoV2373 (Nuvaxovid; Novavax). Zgodnie z aktualnymi rekomendacjami światowych organizacji (WHO, ECDC, EMA), zaleca się szczepienie monowalentnymi preparatami skierowanymi przeciwko dominującemu podwariantowi XBB.1.5. Omicron, które dostępne są także w naszym kraju (jedynie szczepionka NVX-CoV2373 XBB.1.5.) W Polsce podano ponad 58 milionów dawek szczepionek w ciągu 2 lat od ich wprowadzenia [2].

Rozpoczęto liczne badania mające na celu ocenę skuteczności, bezpieczeństwa i tolerancji wymienionych wyżej preparatów. Wykazano, że szczepienia zapewniają ochronę przed ciężkim przebiegiem infekcji SARS-CoV-2 oraz śmiercią, jednak ich skuteczność może być niższa w odniesieniu do pojawiających się z czasem wariantów wirusa, takich jak Delta i Omicron. Gorszy rezultat obserwowano również u osób powyżej 65 roku życia [3]. Pierwotna skuteczność szczepionki (VE) dla preparatu BNT162b2 została oszacowana na ponad 95% w odniesieniu do objawowego zakażenia COVID-19.

Niezależnie od rodzaju szczepionki, u osób z niższym poziomem przeciwciał oraz w wyniku ekspozycji wysokiego ryzyka, występują przełomowe zakażenia [4].

Infekcja SARS-CoV-2 i/lub szczepienie skutkowały immunologiczną odpowiedzią humoralną i powstaniem przeciwciał neutralizujących specyficznych dla SARS-CoV-2, zwłaszcza przeciwko domenie RBD (receptor binding domain) znajdującej się na białku kolca (spike protein - S) [5,6]. Obecność przeciwciał neutralizujących i ich stężenie wydaje się być istotnym markerem ochronnym. Warto wspomnieć, iż WHO wyznaczyło międzynarodowe standardy oceny odpowiedzi humoralnej na szczepienia przeciwko COVID-19. Użycie tych standardów w praktyce klinicznej ma przysłużyć się lepszemu zrozumieniu odpowiedzi immunologicznej, w szczególności w odniesieniu do wartości ochronnych przeciwciał [7,8].

Do oceny skuteczności szczepień na szeroką skalę, potrzebne było narzędzie powszechnie dostępne, mało inwazyjne, o niewielkich wymaganiach dotyczących sprzętu i umiejętności personelu, a także przystępne cenowo. Pomiar poziomu przeciwciał przeciwko białku kolcowemu anty-SARS-CoV-2 IgG wydaje się spełniać wszystkie powyższe wymagania. Przyczyniło się to do wybrania tej metody do badania, którego wyniki znajdują się w dalszej części rozprawy.

Innym aspektem związanym ze szczepieniami, o nie mniejszym znaczeniu klinicznym niż skuteczność, jest bezpieczeństwo. Wiele osób zaszczepionych przyznaje, że doświadczyło działań niepożądanych, w większości łagodnych, o nieznacznym nasileniu, obejmujących podwyższenie temperatury ciała, ból głowy, zmęczenie, bóle mięśniowo-stawowe, dreszcze oraz ból i zaczerwienie w miejscu wkłucia. Czasem poważniejsze skutki szczepień były również obserwowane. Reakcje alergiczne o różnym nasileniu, ze wstrząsem anafilaktycznym włącznie, pierwotna małopłytkowość immunologiczna (ITP), zakrzepica zatok żylnych mózgu (CVST) z występowaniem przeciwciał przeciwko kompleksowi płytkowego czynnika 4 zostały opisane w literaturze medycznej [9]. Rzadkie przypadki zapalenia mięśnia sercowego i osierdzia, głównie dotyczące młodych mężczyzn w wieku 12-39 lat, powiązano z wcześniejszym zastosowaniem szczepionek mRNA [10].

Pomimo niepożądanych skutków szczepienia, których występowanie trudno przewidzieć, wszystkie dostępne szczepionki przeciwko SARS-CoV-2 uznano za bezpieczne, a miliony ludzi na całym świecie zaufało tej opcji zabezpieczenia się przed powikłaniami COVID-19. Do tej grupy zalicza się 298 osób z rejonu Dolnego Śląska, które dobrowolnie zgłosiły się do związanego ze szczepieniami badania poziomu przeciwciał.

IV. Cele rozprawy doktorskiej

Głównym celem badania była ocena odpowiedzi humoralnej na szczepienia u ozdowieńców oraz osób, które wcześniej nie chorowały na COVID-19 oraz ocena długofalowej skuteczności i bezpieczeństwa szczepień.

Podstawowymi założeniami dla poszczególnych prac były:

1. Ocena poziomu przeciwciał anty-SARS-CoV-2 IgG u ozdowieńców i osób wcześniej niezakażonych (naiwnych), planujących zaszczepienie się przeciw SARS-CoV-2.
2. Pomiar stężeń przeciwciał po pierwszej dawce szczepienia, po drugiej dawce/po pełnym cyklu szczepienia (w przypadku 1-dawkowych schematów przewidzianych dla preparatu Ad26.COVS2.S) oraz porównanie ich z wyjściowymi poziomami przeciwciał. Analiza najczęściej występujących poszczepiennych działań niepożądanych, w zależności od zastosowanego preparatu, jego dawki oraz ewentualnej infekcji SARS-CoV-2 w przeszłości.
3. Ponowna ocena poziomu przeciwciał 6 miesięcy po zakończeniu schematu podstawowego szczepienia, z uwzględnieniem skuteczności szczepień (wystąpieniem zachorowań).

W pracy nr 3 chciałam zwrócić szczególną uwagę na ponowną ocenę grupy osób, które miały niższy poziom przeciwciał po drugiej dawce niż przed jej przyjęciem (dane otrzymane i opisane w pracy nr 2). Dodatkowo zapytałam o plany pacjentów wobec dawki przypominającej szczepienia, która w czasie trwania badania miała być udostępniona dla wszystkich zainteresowanych.

VI. Materiały i metody

Wszystkie trzy prace dotyczą pełnoletnich pacjentów z regionu Dolnego Śląska, którzy dobrowolnie zgłosili się do badania przed przyjęciem szczepienia przeciwko SARS-CoV-2. Rekrutacja odbyła się poprzez ogłoszenia w lokalnych mediach, w tym w gazetach, na stronie internetowej szpitala i miała miejsce na początku roku 2021. Po uzupełnieniu przez zainteresowanych formularza kontaktowego, uzupełniałam niezbędne informacje indywidualnie z każdą osobą w celu ustalenia braku przeciwwskazań do udziału w badaniu. Kryteriami wykluczającymi były: cukrzyca, choroba nowotworowa w ciągu ostatnich 5 lat, przewlekłe choroby nerek, wątroby lub płuc, AIDS lub immunosupresja z jakiegokolwiek innego powodu. Kryteria włączenia: ukończenie 18 roku życia, możliwość zbadania poziomu przeciwciał przed przyjęciem pierwszej dawki szczepienia, zaplanowanie daty szczepienia oraz podpisanie pisemnej zgody na udział w projekcie badawczym.

Do pomiaru poziomu przeciwciał wykorzystano test QuantiVac anti-SARS-CoV-2 IgG ELISA (EUROIMMUN, Luebeck, Germany). Wyniki zostały podane zgodnie z zaleceniami producenta, w jednostkach międzynarodowych IU/ml. Pierwotnie do badania zostało zakwalifikowanych 298 osób, 171 ozdowieńców (grupa I) i 127 naiwnych pacjentów (grupa II).

Zarejestrowane osoby zgłaszały się w wyznaczonym terminie na pobranie pierwszej próbki krwi do oceny poziomu przeciwciał oraz wypełniały ankietę dotyczącą ewentualnej infekcji SARS-CoV-2 w przeszłości i ciężkości objawów zakażenia, ogólnego stanu zdrowia, wcześniejszych reakcji alergicznych na szczepienia lub inne substancje. Przebieg COVID-19 było weryfikowane poprzez przedstawienie dodatniego wyniku badania PCR, testu antygenowego lub serologicznego w kierunku SARS-CoV-2 lub innych czynników silnie sugerujących przebieg COVID-19 (np. utrata węchu lub smaku podczas mieszkania z osobą z potwierdzoną infekcją). Na tym etapie badania, szczepienia były częściowo dostępne, tzn. najpierw dla poszczególnych grup zawodowych, następnie zależnie od roku urodzenia. W związku z częstymi zmianami w zasadach zapisywania się na szczepienia, a także z powodu indywidualnych przeciwwskazań do szczepień w danym dniu, odstęp między pobraniem krwi do badań, a faktycznym przyjęciem szczepienia wahał się od 1 dnia do 6 tygodni i średnio wynosił 1 tydzień. Preparaty, którymi pacjenci mieli być szczepieni, były wybierane w

momencie zapisywania się, zależnie od dostępności (BNT162b2, mRNA-1273, ChAdOx1 nCoV-19 i Ad26.COV2.S). Ten etap badania odbywał się w terminie 20 lutego – 19 maja 2021 r.

Na kolejne pobranie krwi pacjenci zgłaszali się przed i po drugiej dawce szczepienia. Schematy szczepień poszczególnych producentów przewidywały zróżnicowane odstępy między pierwszą i drugą dawką (od 21 dni do 3 miesięcy), dlatego w celu ujednolicenia zasad badania poziomu przeciwciał, drugie pobranie krwi odbywało się 1-7 dni przed drugą dawką, a trzecie około miesiąc po zakończeniu pełnego schematu szczepienia (po drugiej dawce lub po szczepieniu Ad26.COV2.S). Na poszczególnych etapach badania zostało zbadanych:

D0 (przed szczepieniem) = 298 osób (grupa I: 171; grupa II: 127)

D1 (po pierwszej dawce/przed drugą dawką) = 286 (I: 163, II: 123)

D2 (po drugiej dawce) = 295 (I: 169, II: 126)

Zdecydowana większość przyjęła szczepionki mRNA (BNT162b2 n=191, mRNA-1273 n= 70). Pozostałe osoby zostały zaszczepione przy użyciu preparatów ChAdOx1 nCoV-1 (n= 25) lub Ad26.COV2.S (n=9). Próbkę krwi do badań zostały pobrane do 25 sierpnia 2021 r.

Porównując wyniki z etapów D1 i D2, u części pacjentów zaobserwowano kontrintuicyjny spadek stężenia przeciwciał po otrzymaniu drugiej dawki. Stało się to przyczyną zaproszenia wolontariuszy na kolejne badanie, około 6 miesięcy po zakończeniu szczepień. Choć zjawisko to wystąpiło niezależnie od rodzaju przyjętego preparatu, w celu ograniczenia czynników mogących wpływać na wyniki, do tej części zrekrutowano osoby, które otrzymały 2 dawki BNT162b2. Łącznie 87 pacjentów podzielono na 3 podgrupy:

A: ozdrowieńcy, u których wyjściowo obecne były przeciwciała anty-SARS-CoV-2 IgG, a w wyniku szczepienia nastąpił wzrost ich poziomu (27 osób)

B: naiwni, u których wyjściowo nie wykryto obecności przeciwciał (30 osób)

C: ozdrowieńcy, u których wyjściowo obecne były przeciwciała, ale po otrzymaniu drugiej dawki szczepienia poziom przeciwciał był niższy niż przed jej podaniem (30 osób)

Jednocześnie z ustaleniem terminu zgłoszenia się do badania, pacjenci byli proszeni o wypełnienie ankiety dotyczącej wieku, wzrostu, wagi, przebytych chorób od czasu ostatniego szczepienia (w tym infekcji SARS-CoV-2), szczepienia przeciwko grypie, palenia papierosów. Badanie wykonano w okresie 17 listopada – 15 grudnia 2021 r.

Wyniki uzyskanych badań poddano opracowaniu statystycznemu. Dla wszystkich grup zostały wyliczone liczba przypadków (N), średnia, odchylenie standardowe (SD), mediana, zakres (min-max) oraz dolny i górny kwartył (25Q-75Q) badanych parametrów ilościowych. Zmienne jakościowe przedstawiono jako wartości bezwzględne i odsetki (%). Normalność rozkładu sprawdzano testem Shapiro-Wilka, a jednorodność wariancji testem Levene'a. Weryfikację hipotezy o równości średnich parametrów w grupach niezależnych o jednorodnej wariancji przeprowadzono metodą analizy wariancji ANOVA lub dla grup o niejednorodnej wariancji testem nieparametrycznym U Mann-Whitney'a (dla dwóch grup) lub Kruskala-Wallisa (dla trzech i więcej grup). Dla parametrów jakościowych częstość występowania cechy w grupach analizowano testem χ^2_{df} z odpowiednią liczbą stopni swobody df ($df=(m-1)*(n-1)$, gdzie m – liczba wierszy, n – liczba kolumn). $P \leq 0.05$ uznawano za znaczące statystycznie. Analizę statystyczną przeprowadzono wykorzystując komputerowe pakiety programów statystycznych EPIINFO Ver. 7.2.4.0 oraz Statistica Ver. 13.3.

VII. Podsumowanie wyników

Wynikiem pierwszej pracy jest opis sytuacji epidemiologicznej sprzed wprowadzenia powszechnego dostępu do szczepień przeciwko SARS-CoV-2. Do badania zakwalifikowano 298 osób, które podzielono na 2 podstawowe grupy: grupę I – ozdowieńców oraz II – osób naiwnych. Nie wykazano statystycznie istotnych różnic względem wieku ($p = 0,177$), ani płci ($p = 0,828$). Po wykonaniu pierwszych badań, wśród pacjentów grupy I zdołano wyodrębnić specyficzne ze względu na poziomy przeciwciał, 3 podgrupy: podgrupa I – pacjenci posiadający przeciwciała, którzy przebyli zakażenie SARS-CoV-2 asymptotycznie (bez wcześniejszej wiedzy o zakażeniu, $n = 21$, co stanowi 12,28 % grupy I i 7,05% całej grupy badanej), podgrupa II – ozdowieńcy objawowi, u których nie wykryto obecności przeciwciał anti-SARS-CoV-2 IgG ($n = 23$, 13,45% grupy I i 7,72% całej grupy), podgrupa III – ozdowieńcy z potwierdzoną infekcją SARS-CoV-2 w przeszłości, z dodatnim wynikiem przeciwciał anti-SARS-CoV-2 IgG ($n = 127$). Podgrupa I składała się z osób w wieku 24-50 lat, ich mediana poziomu przeciwciał wynosiła 126,4 IU/ml (IQR 54,4 – 200,0 IU/ml). Po otrzymaniu wyników upewniono się, iż uczestnicy nie pamiętali żadnych objawów mogących odpowiadać COVID-19. Seronegatywni ozdowieńcy (podgrupa II) w wieku 23-63 lata zostali zbadani na obecność przeciwciał anti-SARS-CoV-2 2,5-6,5 miesiąca od czasu zachorowania. Pacjenci podgrupy III w wieku 21-69 lat uzyskali medianę przeciwciał 123,3 IU/ml (IQR 58,7 – 252,8 IU/ml). Odstęp czasowy pomiędzy pobraniem krwi do badań, a objawami COVID-19 wynosił od 18 dni do 7 miesięcy.

Szczegółowa analiza podgrup grupy I wykazała znaczące różnice w wieku pacjentów. W podgrupie III (seropozytywni ozdowieńcy) wiek był istotnie wyższy niż w podgrupie II (seronegatywni ozdowieńcy): mediana odpowiednio 45,0 lat [IQR 40,0-52,0] vs. 43,0 lat [IQR 33,0-46,0], $p = 0,0271$. Podobną zależność można zaobserwować w porównaniu podgrupy III z grupą II (pacjentów naiwnych): mediana odpowiednio 45,0 lat [IQR 40,0-52,0] vs. 43,0 lat [IQR 39,0-47,0], $p = 0,0228$. Nie wykazano takich różnic między podgrupą III, a I ($p = 0,0983$).

W drugiej pracy przedstawiono wyniki badania 295 pacjentów, którzy zgłosili się ponownie po pierwszej i drugiej dawce szczepienia. Odpowiednio 286 osób (grupa I: 163, grupa II: 123) zostało przebadanych pod kątem obecności przeciwciał anti-SARS-CoV-2 IgG w czasie 1-7

dni przed drugą dawką szczepienia oraz 295 osób (I: 169, II: 126) około miesiąc po jej przyjęciu. Szczegółowa charakterystyka grup znajduje się w tabeli 1.

Tabela 1. Charakterystyka grupy badanej.

Parametry		Grupa I (n=171)	Grupa II (n=127)	Wartość p
Wiek	Średnia $\pm$ SD	44.0 $\pm$ 9.4	42.6 $\pm$ 7.2	0.177
	Me [IQR]	44.0 [39.0 - 49.0]	43.0 [39.0 – 47.0]	
płeć	żeńską	101 (57.81%)	74 (58.27%)	0.828
	męską	70 (42.19%)	53 (41.73%)	
Poziom przeciwciał anty-SARS-CoV-2 [IU/ml]	Przed szczepieniem (średnia $\pm$ SD)	190.3 $\pm$ 328.4	0	<0.001
	Me [IQR]	105.6 [38.4-198.4]	0	
Stwierdzona infekcja SARS-CoV-2 w przeszłości	tak	150	0	
	nie	21	127	
Rodzaj szczepienia na etapie D2	BNT162b2	104	87	
	mRNA-1273	37	33	
	ChAdOx1 nCoV-19	21	4	
	Ad26.COV2.S	6	3	

Grupa I- ozdrowieńcy, grupa II – osoby naiwne, SD – odchylenie standardowe, Me – mediana, IQR – rozstęp ćwiartkowy, etap D2 – badanie po drugiej dawce szczepienia/ po jednej dawce Ad26.COV2.S

Po pierwszej dawce 2 osoby z grupy I i 2 z grupy II nie posiadały przeciwciał anty-SARS-CoV-2, jednak po otrzymaniu drugiej dawki 100% uczestników badania odpowiedziało na szczepienie.

- Pacjent 1 (grupa I) – mężczyzna, 27 lat, COVID-19 w przeszłości, poziomy przeciwciał: D0 = 57,6, D1 = 0, D2 = 2860 IU/ml

- Pacjent 2 (grupa I) – kobieta, 39 lat, COVID-19 w przeszłości, poziomy przeciwciał: D0 = 0, D1 = 0, D2 = 1318 IU/ml
- Pacjent 3 (grupa II) – mężczyzna, 61 lat, poziomy przeciwciał D0 = 0, D1 = 0, D2 = 1050 IU/ml
- Pacjent 4 (grupa II) – kobieta, 38 lat, poziomy przeciwciał D0 = 0, D1 = 0, D2 = 2464 IU/ml

U obu grup zaobserwowano znaczny wzrost poziomu przeciwciał po pierwszej dawce – dla grupy I był to 52-krotny wzrost. Dla grupy II, po drugiej dawce, zaobserwowano prawie 5-krotny wzrost miana przeciwciał. Grupa ozdowieńców prezentowała istotnie wyższe poziomy przeciwciał w porównaniu do grupy II (tabela 2). Z tego względu porównano wyniki grupy I po pierwszej dawce z wynikami grupy II po drugiej dawce, traktując szczepienie w przypadku ozdowieńców niczym dawkę przypominającą. Immunologiczna stymulacja grupy I była silniejsza niż grupy II (mediana 4736,0 vs. 3056,0 IU/ml; $p = 0,00003$).

Tabela 2.

	Poziomy przeciwciał anty-SARS-CoV-2 IgG [IU/ml]					
	D0- przed szczepieniem		D1-po pierwszej dawce		D2 – po drugiej dawce	
	Średnia ± SD	mediana [IQR]	Średnia ± SD	Me [IQR]	Średnia ± SD	Me [IQR]
Grupa I	190,3± 328,4	105,6 [38,4; 198,4]	5501,5±4380,0	4736,0 [2676,9; 6144,0]	5523,7± 4016,4	4560,0 [3040,0; 7232,0]
Grupa II	0,00±0,00	0,0 [0,0; 0,0]	751,9± 897,7	451,2 [217,6; 915,2]	3625,6± 2568,9	3056,0 [2048,0; 4512,0]
Wartość p	0,00000		0,00000		0,00000	

SD – odchylenie standardowe, Me – mediana, IQR – rozstęp ćwiartkowy

Szczególną uwagę wśród grupy I zwróciło 61 osób (37,7% grupy I), których poziomy przeciwciał po drugiej dawce okazały się niższe niż przed jej przyjęciem ($D2 < D1$). Analiza danych dotyczących tej podgrupy oraz pozostałych ozdowieńców wykazała, że tendencje

spadkowe dotyczyły osób ze szczególnie wysokimi stężeniami przeciwciał po pierwszej dawce ($p = 0,0001$). Możliwy wpływ mógł mieć również wiek – w podgrupie D2>D1 znajdowali się młodszy uczestnicy (średnia wieku 42,3 vs. 45,4 lat; $p = 0,0418$). Zjawisko występowało niezależnie od zastosowania jednej z trzech dwudawkowych szczepionek (BNT162b2, mRNA-1273, ChAdOx1 nCoV-19).

Wszyscy uczestnicy badania zostali zaszczepieni preparatami dwu- lub jedno-dawkowymi (Ad26.COVS.2), przy czym pierwsza i druga dawka były jednakowe. Jedna osoba pierwotnie naiwna zachorowała na COVID-19 po przyjęciu pierwszej dawki mRNA-1273, poza tym nikt nie uległ objawowemu zakażeniu w ciągu 4 tygodni od zakończenia podstawowego schematu szczepienia. Najwyższe poziomy przeciwciał należały do osób zaszczepionych preparatami mRNA-1273, podobne wyniki gwarantował BNT162b2.

Niektóre działania niepożądane szczepień udało się powiązać z następowymi wyższymi poziomami przeciwciał. Spośród objawów raportowanych przez grupę I po pierwszej dawce była to gorączka ($p = 0,0222$), po drugiej dawce natomiast gorączka ($p = 0,00002$), osłabienie ($p = 0,0$), ból w miejscu wkłucia ($p = 0,0161$), bóle mięśniowo-stawowe ($p = 0,0221$). Podobne spostrzeżenia dotyczą grupy II, której zgłaszane działania niepożądane po drugiej dawce pozytywnie korelowały z poziomem przeciwciał: gorączka ($p = 0,00026$), ból w miejscu wkłucia ($p = 0,00515$), osłabienie ($p = 0,0114$), bóle mięśniowo-stawowe ($p = 0,046$).

W pracy nr 3 skontrolowano poziomy przeciwciał 87 osób, wcześniej zaszczepionych dwoma dawkami BNT162b2, po około 6 miesiącach od przyjęcia drugiej dawki. Bazując na wynikach poprzednich etapów badania, uczestników podzielono na podgrupy: A - ozdrowieńcy, u których wyjściowo obecne były przeciwciała anty-SARS-CoV-2 IgG, a w wyniku szczepienia nastąpił wzrost ich poziomu (27 osób); B - naiwni, u których wyjściowo nie wykryto obecności przeciwciał (30 osób); C - ozdrowieńcy, u których wyjściowo obecne były przeciwciała, ale po otrzymaniu drugiej dawki szczepienia poziom przeciwciał był niższy niż przed jej podaniem (30 osób). Żadna z powyższych osób nie przeżyła objawowej infekcji COVID-19 w ciągu 6 miesięcy od szczepienia.

Poszukując czynników mogących mieć wpływ na utrzymujące się poziomy przeciwciał, uchwycono powiązanie ich z wyjściowymi poziomami przeciwciał (D0 & D3, $p = 0,000$). Badana grupa prezentowała nieco podwyższony wskaźnik BMI lub znajdujący się w górnej granicy normy i choć wydaje się to rzutować na wyjściowo wyższe poziomy przeciwciał

(dla D0 $p = 0,001$), nie wykazano związku nadwagi z poziomami przeciwciał w czasie i po szczepieniu.

Przed szczepieniem (D0) różnice pomiędzy podgrupami ozdrowieńców i pacjentów naiwnych były wyraźnie widoczne (A vs. B $p = 0,0$; B vs. C $p = 0,0$; A vs. C $p = 0,353$). Po pierwszej dawce (D1) różnice pozostały podobne, jednak po drugiej dawce (D2) zaczęto obserwować zmiany w wynikach części ozdrowieńców, u których pojawiła się tendencja spadkowa poziomu przeciwciał (A vs. B $p = 0,0$; B vs. C $p = 0,633$; A vs. C $p = 0,001$). W czasie ostatniej analizy 6 miesięcy po szczepieniu różnice utrzymały się, jednak charakterystyka grup była bardziej zbliżona do wyjściowych danych na etapie D0 i D1 (A vs. B $p = 0,0$; B vs. C $p = 0,007$; A vs. C $p = 0,048$).

Podgrupa C uzyskała wyższe wyniki poziomu przeciwciał w porównaniu z podgrupą A na etapie D0 i D1, a także z podgrupą B na etapie D2, jednak nie wykazano statystycznej istotności tych różnic (odpowiednio $p = 0,353$; $p = 0,0733$; $p = 0,633$). 6 miesięcy po szczepieniu podgrupa C charakteryzowała się wciąż wysokimi stężeniami przeciwciał, wyższymi niż podgrupa B ($p = 0,007$), ale niższymi niż podgrupa A ($p = 0,048$). Analizując spadki obserwowane po 6 miesiącach w każdej z poszczególnych grup, można zauważyć, że podgrupa A utraciła z biegiem czasu więcej przeciwciał niż podgrupy B i C (mediana wartości D2-D3 dla A: 5389 IU/ml, dla B: 3105 IU/ml, dla C: 3158 IU/ml; $p = 0,003$), a podgrupa B charakteryzuje się największą częstością zmian – 4,5-krotnie niższymi wynikami.

Zarówno palenie papierosów, jak i szczepienie przeciwko grypie w sezonie 2021/2022 nie miały wpływu na stężenie przeciwciał abty-SARS-CoV-2 IgG na tym etapie badania (odpowiednio $p = 0,300$ i $p = 0,243$).

W związku z wprowadzeniem powszechnej możliwości przyjęcia trzeciej (przypominającej) dawki szczepienia w trakcie trwania badania, pacjenci zostali zapytani, czy planują zaszczepienie się. Większość osób deklarowała zapisanie się na szczepienie w rekomendowanym czasie 6-12 miesięcy od poprzedniej dawki (A-15; B-21; C-17; 53/87 osób, co daje 60,91%), jednak interesująco dużo uczestników uzależniało decyzję od aktualnego na tamten moment poziomu przeciwciał (A-11; B-7; C-12; 30/87, co daje 34,48%).

VIII. Dyskusja

Wobec niespotykanej od dawna skali problemu, jakim jest pandemia COVID-19, szczepienia przeciwko SARS-CoV-2 dla wielu były najbardziej wyczekiwany osiągnięciem współczesnej medycyny. Jak wynika również z niniejszej rozprawy, ochrona przed infekcją SARS-CoV-2 jest szczególnie ważna dla osób w wieku ≥ 50 lat, u których ryzyko ciężkiego przebiegu COVID-19 jest wyższe [11]. Do szybkiego wzrostu odnotowywanych nowych przypadków infekcji niewątpliwie przyczyniła się zdolność zakażenia osób bezobjawowych. Dotyczy to nie tylko osób, które dopiero miały rozwinąć objawy, ale także tych, które przeszły infekcję asymptomatycznie [12,13]. Nieco ponad 12% badanych przeze mnie ozdrowieńców doświadczyło nieświadomie infekcji, będąc potencjalnym źródłem SARS-CoV-2 dla osób z najbliższego otoczenia. Występowanie objawów, a także ich ciężkość jest także związana ze stężeniem przeciwciał, które układ odpornościowy organizmu produkuje w późniejszym czasie [14]. ozdrowieńcy, którzy osiągnęli graniczne poziomy przeciwciał, nie prezentowali objawów lub chorowali skąpo objawowo, natomiast pacjent z najwyższym wynikiem był hospitalizowany z powodu ciężkiego przebiegu COVID-19. Czas, który minął od infekcji do testowania krwi z pewnością wpłynął na prezentowane wyniki – wiadomym jest, iż poziom przeciwciał jest względnie stabilny jedynie przez kilka miesięcy, zależnie od różnych czynników osobniczych i środowiskowych [15]. Łącznie z teorią o występowaniu wśród pacjentów osób niereagujących na działanie substancji immunizujących (ang. non-responders), może to wyjaśniać fakt znalezienia się w grupie badanej 23 seronegatywnych ozdrowieńców [16].

U ozdrowieńców, w porównaniu do grupy pacjentów naiwnych, obserwowano znacząco wyższe poziomy anty-SARS-CoV-2 IgG i podobne dane prezentowane są przez innych autorów [17]. Pierwotna infekcja dla układu immunologicznego może być porównana do pierwszej dawki szczepionki, wówczas samo szczepienie byłoby podobne do dawki przypominającej. Czasowa odporność przeciwko SARS-CoV-2 nabyta drogą naturalną może sprawiać, że ozdrowieńcy po pierwszym szczepieniu prezentują porównywalną lub silniejszą humoralną odpowiedź immunologiczną w porównaniu do osób naiwnych nawet po drugiej dawce szczepienia [18,19]. Rodzi to pytanie o zasadność drugiej dawki schematu podstawowego u osób, które już przebyły zakażenie SARS-CoV-2. Inną opcją niż redukcja schematu podstawowego jest wydłużanie odstępu między dawkami, co może nie tylko wzmocnić odpowiedź immunologiczną, ale także zmniejszyć ryzyko ciężkich powikłań poszczepiennych

[20]. Dylemat pozostaje aktualny także w chwili obecnej, kiedy COVID-19 wywołuje głównie podwariant Omicron XBB.1.5, a dostępne dawki przypominające są dedykowane właśnie jemu; ponadto w skali globalnej wciąż wiele osób nie zostało uodpornionych ani jedną dawką szczepienia.

Wśród uczestników badania 100% odpowiedziało na pełny schemat szczepienia, jednak wyniki 4 osób (1,4% z grupy 286 osób) po pierwszej dawce okazały się być równe zero. Dane te są porównywalne z innymi doniesieniami, takimi jak praca G. Iacobucciego, który przeanalizował 8517 przypadków pacjentów z Anglii i Walii, szczepionych pierwszą dawką, z czego 96,42% wytworzyło przeciwciała przeciwko białku kolca [21]. Mimo bardzo dobrej ogólnej odpowiedzi immunologicznej, u 61 ozdowieńców wykazano tendencję spadkową przeciwciał po drugiej dawce, w porównaniu do wyników przed jej podaniem. Warto zauważyć, że wśród tych pacjentów obserwowano zasadniczo wyższe poziomy przeciwciał, które mogły blokować produkcję antygenów SARS-CoV-2, osłabiając tym samym efektywność komórek prezentujących antygen i prowadząc do ograniczonej odpowiedzi humoralnej po drugiej dawce [22]. Niektórzy autorzy sugerują, że to zjawisko może być reakcją na glikol polietylenowy (PEG) obecny w szczepionce BNT162b2, jednak wśród 61 pacjentów tylko 39 było zaszczepionych z użyciem właśnie tego preparatu (16 osób otrzymało preparaty mRNA-1273, a 6 ChAdOx1 nCoV-19). Innym wyjaśnieniem może być fenomen „immune imprinting”, czyli immunologicznego wdrukowywania. Po raz pierwszy opisany w 1947 roku w odniesieniu do odpowiedzi immunologicznej po zachorowaniu na grypę, mechanizm może dotyczyć różnych wirusów [23]. Powszechnie występujące infekcje sezonowymi koronawirusami mogły wpłynąć na późniejszą odpowiedź na szczepienia przeciwko SARS-CoV-2 [24]. Zjawisko zostało już wcześniej powiązane także ze szczepionkami przeciwko SARS-CoV-2, przed dominacją wariantu Omicron [25].

Wszystkie cztery rodzaje szczepionek wykazały zadowalającą odpowiedź humoralną. Najwyższe poziomy przeciwciał po drugiej dawce szczepienia osiągnęli pacjenci, którzy otrzymali preparaty mRNA-1273, jednak obie dostępne szczepionki mRNA cechowały się zbliżonymi wynikami. Również raportowane przez uczestników badania objawy niepożądane o niewielkim nasileniu dotyczyły najczęściej szczepionki mRNA-1273 i są porównywalne z innymi doniesieniami [26]. Technologia mRNA pozostaje istotnym i użytecznym osiągnięciem nowoczesnej nauki. Z powodu stosunkowo niewielkiej ilości uczestników poddanych szczepieniu specyfikami wektorowymi ChAdOx1 nCoV-1 i Ad26.COV2.S, nie można wyciągnąć podobnych wniosków dla tych preparatów.

Na kolejnym etapie badania 6 miesięcy po zakończeniu wakcynacji, celowo ocenione zostały osoby po otrzymaniu jedynie preparatu BNT162b2, aby móc zweryfikować kolejną kwestię dotyczącą szczepień – czy przejściowy spadek poziomu przeciwciał u ozdowieńców po drugiej dawce miał swoje dalsze konsekwencje? Udało się potwierdzić długofalową skuteczność u 87 uczestników (w tym u 30 osób z udokumentowaną tendencją spadkową poziomu przeciwciał) – nikt z badanej grupy nie przeszedł w tym czasie COVID-19. Nie znaleziono istotnych różnic w stężeniach przeciwciał u wspomnianych 30 osób i innych badanych ozdowieńców.

Choć otyłość staje się coraz bardziej powszechnym problemem zdrowotnym również w Polsce, omawiane przeze mnie wyniki dotyczą pacjentów z prawidłowym wskaźnikiem BMI lub nadwagą (przedział 25,0-29,9). Zaobserwowano wyższe stężenia przeciwciał u osób z nadwagą, co może wiązać się ze względnie cięższym, bardziej objawowym przebiegiem infekcji SARS-CoV-2. Niewiele jest doniesień w literaturze odnośnie wpływu samej nadwagi na odpowiedź humoralną i częściej jest wiązana z niższymi poziomami przeciwciał niż u osób z prawidłowym BMI [27]. Jest to temat wymagający dalszych badań, jako że znajomość każdego czynnika mogącego wpływać na odpowiedź poszczepienną przeciwko SARS-CoV-2, wirusowi dobrze zdomowionemu w realiach współczesnej ochrony zdrowia, zbliża nas do lepszego zrozumienia zasad skutecznej walki z COVID-19.

IX. Wnioski

Szczepienie przeciw COVID-19, niezależnie od rodzaju zastosowanej szczepionki, u wszystkich pacjentów było skuteczne i indukowało odpowiedź humoralną. Objawy niepożądane, które wystąpiły u części osób szczepionych, nasilające się po kolejnej dawce antygenu (wirus lub szczepionka), nie miały istotnego wpływu na stan zdrowia.

Wnioski z publikacji nr 1:

- Objawy COVID-19 były częściej obserwowane u starszych pacjentów, a ciężkość objawów może być powiązana z wyższymi stężeniami przeciwciał anti-SARS-CoV-2
- Część pacjentów może nie prezentować objawów infekcji SARS-CoV-2, pozostając przy tym źródłem zakażenia dla zdrowych osób z otoczenia
- Obecność lub brak przeciwciał anti-SARS-CoV-2 IgG nie może być jedynym czynnikiem determinującym stwierdzenie zakażenia SARS-CoV-2 w przeszłości

Wnioski z publikacji nr 2:

- Wszystkie cztery rodzaje szczepień spowodowały odpowiedź humoralną zarówno u ozdowieńców jak i pacjentów naiwnych, ze szczególnym wskazaniem na preparaty mRNA (mRNA-1273 oraz BNT162b2)
- U ozdowieńców po przyjęciu pierwszej dawki zaobserwowano istotnie wyższe poziomy przeciwciał niż u osób naiwnych po drugiej dawce, co świadczy o silniejszym pobudzeniu immunologicznym
- Wśród ozdowieńców zaszczepionych preparatami mRNA-1273, BNT162b2 i ChAdOx1 nCoV-19, 37,7% wykazało spadek poziomu przeciwciał po drugiej dawce w porównaniu do wyników sprzed jej przyjęcia, co może wskazywać na zasadność wydłużania interwału czasowego między kolejnymi dawkami u ozdowieńców. Takie postępowanie może spowodować rzadsze występowanie ciężkich powikłań poszczepiennych. Z kolei osoby starsze i takie, u których występują niskie stężenia przeciwciał powinny być zachęcane do przyjęcia dawek przypominających
- W grupie naiwnej, objawy niepożądane były silniej wyrażone po przyjęciu drugiej dawki szczepienia, podczas gdy u ozdowieńców były często obserwowane już po pierwszej dawce

Wnioski z publikacji nr 3:

- Niezależnie od stężenia przeciwciał uzyskanych na wcześniejszych etapach badania, żaden pacjent nie manifestował objawów infekcji SARS-CoV-2 w ciągu 6 miesięcy od przyjęcia drugiej dawki szczepienia
- Czasowy spadek poziomu przeciwciał u części ozdowieńców po drugiej dawce nie miał istotnego wpływu na wyniki badań po 6 miesiącach
- Nadwaga u pacjentów chorujących na COVID-19 może wpływać na wyższe poziomu przeciwciał, jednak nie wykazano takiego związku z poziomem przeciwciał po szczepieniu

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XI. Publikacje stanowiące podstawę rozprawy doktorskiej.

Research letters

An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland

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Abstract

Background. Since the beginning of the coronavirus disease (COVID-19) pandemic, numerous infections have been observed with various symptoms and degrees of severity. Not all patients have had a confirmation of infection made using reverse transcription polymerase chain reaction (RT-PCR) or antigen tests. It has been observed that some people, including convalescents or those without knowledge of a past infection, perform serological tests to detect anti-severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antibodies.

Objectives. We aimed to evaluate the levels of anti-SARS-CoV-2 immunoglobulin G (IgG) antibodies in a cohort of convalescents and in individuals not previously infected, who were willing to get vaccinated. We also aimed to assess several socio-clinical factors associated with participants' humoral responses.

Materials and methods. We recruited 298 individuals from the region of Lower Silesia who were willing to get vaccinated for SARS-CoV-2. The participants were divided into 2 groups: convalescents (group I) and participants without a past infection (group II). Several seropositive individuals in group II were identified, and they were transferred to group I, resulting in a final distribution of 171 individuals in group I and 127 individuals in group II. For serological testing, the QuantiVac anti-SARS-CoV-2 (IgG) enzyme-linked immunosorbent assay (ELISA) was used.

Results. The results showed the presence of anti-SARS-CoV-2 IgG antibodies in participants from group I, with an average number of 190.3 IU/mL. Twenty-three participants (13.45%) did not have a detectable level of antibodies despite a previous SARS-CoV-2 infection. In 21 participants (12.28%), antibodies were detected despite no previous symptoms of infection (average level: 145.0 IU/mL).

Conclusions. Older participants were more likely to experience a symptomatic SARS-CoV-2 infection, and the severity of the symptoms was related to higher antibody titers seen later after COVID-19. Numerous individuals from group II were unaware of past SARS-CoV-2 infections. In several participants, antibodies were not detected despite a previous infection.

Key words: antibodies, serology, seronegative, SARS-CoV-2, asymptomatic

Background

The first information about severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) emerged at the beginning of 2020. At present, over 12.88 billion vaccine doses have been administered worldwide, with 67.9% of the world's population having received at least 1 dose. In Poland, over 57 million vaccines were administered in the 2 years since their introduction.¹ However, we are still facing new infections in everyday clinical practice. Therefore, we are continuously in need of information regarding this topic.²

Serological tests have been used to detect antibodies produced as a result of infection. Severe disease, compared to mild disease, correlates with persistently higher antibody levels.³ There is also a small group of people, mainly those with mild/asymptomatic infections, who do not produce antibodies.⁴ However, it should be noted that the detection of the persistence of antibodies can vary depending on the assay used.⁵

Objectives

We present preliminary data on a cohort of participants qualified to receive vaccination against SARS-CoV-2. This is the 1st part of a larger study evaluating the humoral immune response to vaccination against coronavirus disease (COVID-19). The 2nd part of the analysis, entitled "How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection" has also been published (*Vaccines*. 2022;10(7):1042) and is available online (<https://doi.org/10.3390/vaccines10071042>).

The main aim of this study is to evaluate the levels of anti-SARS-CoV-2 immunoglobulin G (IgG) antibodies in a cohort of convalescents and not previously infected individuals from the Lower Silesia region (Poland) who were willing to get vaccinated.

Materials and methods

The inclusion criteria were: age >18 years, providing written informed consent to participate in the study and a willingness to get vaccinated. Two groups of participants were selected for the study: COVID-19 convalescents (group I) and naïve participants (group II). The exclusion criteria were: the presence of diabetes, any cancer detected within the last 5 years, chronic kidney, liver or lung diseases, acquired immunodeficiency syndrome (AIDS), or immunosuppression for any other reason.

Before each blood draw, the participants were asked to complete a questionnaire. The questions concerned previous SARS-CoV-2 infections and whether it was confirmed through testing, as well as general wellbeing, persistence of COVID-19 symptoms, adverse vaccine reactions, chronic diseases, and allergic reactions to drugs, substances and foods.

Overall, 298 participants were included in the study. After receiving results showing that 21 supposedly naïve participants had current IgG antibodies, we decided to analyze them together with the convalescent group, which resulted in the final division: group I (COVID-19 convalescents, $n = 171$) and group II (naïve participants, $n = 127$).

This single-center study was conducted from February 20, 2021, to May 19, 2021. The participants were inhabitants of the Lower Silesia region, aged 21–69 years, and were of both sexes. The participants were recruited by announcements in the local media. Due to changes in the registration rules and participants' individual contraindications at the moment, the interval between taking the blood sample for testing and the actual vaccination date varied from 1 day to 6 weeks, usually approx. 1 week (mean: 2.00, interquartile range (IQR): 0.25–6.00, standard deviation (SD): 8.54). Before vaccination, all participants were tested for the presence of anti-SARS-CoV-2 IgG antibodies.

Plasma samples were collected using heparin, centrifuged, and stored in aliquots at -70°C for later use. The QuantiVac anti-SARS-CoV-2 (IgG) enzyme-linked immunosorbent assay (ELISA) (EUROIMMUN, Lübeck, Germany) was used for quantitative detection of anti-SARS-CoV-2 antibodies by means of 6-point calibration curve.

The ELISAs were performed and the results were evaluated as recommended by the manufacturer. Samples with an absorbance higher than the absorbance of the highest standard (386 international units (IU)/mL) were diluted and retested. The assay was standardized against the First WHO International Standard for anti-SARS-CoV-2 immunoglobulin (NIBSC 20/136) and the quantitative results are given in standardized units (IU/mL).

Ethical approval

This study received approval from the Bioethics Committee of Wrocław Medical University, Poland (approval No. 51/2021). The study was performed in accordance with the Declaration of Helsinki and the principles of good clinical practice with respect to the rights and dignity of participants.

Statistical analyses

Counts, percentages, means, medians, SDs, ranges, and lower and upper quartiles are reported where appropriate. The normality of the distributions was tested with the Shapiro–Wilk test.

Statistical significance between means for different groups was calculated using the non-parametric Kruskal–Wallis test, followed by Dunn's post hoc tests with Bonferroni correction. Statistical significance between frequencies was calculated using the χ^2 test.

Table 1. Characteristics of coronavirus disease (COVID-19) convalescents (group I) and naïve participants (group II)

Parameters		Group I convalescents (n = 171)	Group II (n = 127)	p-value
Age [years]	M ±SD	44.0 ±9.4	42.6 ±7.2	0.177 Mann-Whitney U test, U = 9715.0
	median (IQR)	44.0 (39.0–49.0)	43.0 (39.0–47.0)	
Sex, n (%)	female	101 (57.81)	74 (58.27)	0.828 $\chi^2 = 0.04$
	male	70 (42.19)	53 (41.73)	
Previous allergic reactions to vaccines, n (%)	yes	3 (1.75)	1 (0.79)	0.639 Fisher's exact test
	no	168 (98.25)	126 (99.21)	
Musculoskeletal pain, n (%)	yes	4 (2.34)	0 (0)	0.139 Fisher's exact test
	no	167 (97.66)	127 (100)	
Fatigue, n (%)	yes	9 (5.26)	0 (0)	0.012 Fisher's exact test
	no	162 (94.74)	127 (100)	
Taste and smell loss, n (%)	yes	15 (8.77)	0 (0)	0.002 $\chi^2 = 10.03$
	no	155 (90.64)	127 (100)	
Anti-SARS-CoV-2 antibodies level [IU/mL]	before vaccination, M ±SD	190.3 ±328.4 (n = 148)	0 (n = 127)	<0.001 Mann-Whitney U test, U = 1512.0
	median (IQR)	105.6 (38.4–198.4)	0	

SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; M ±SD – mean ± standard deviation; IQR – interquartile range.

A value of $p < 0.05$ was required to reject the null hypothesis. Statistical analyses were performed using the Statistica v. 13.3 (StatSoft Inc., Tulsa, USA) software package.

Results

All 298 participants were tested for anti-SARS-CoV-2 IgG antibodies, creating 2 groups: I (COVID-19 convalescents, $n = 171$) and II (naïve participants, $n = 127$). Tables 1,2 present characteristics of the groups.

After testing the blood samples, the results showed that anti-SARS-CoV-2 IgG antibodies were present in participants from group I with a median of 105.6 (38.4–198.4) IU/mL. In group II, no spike antibodies were found (0 IU/mL). The detailed data are presented in Fig. 1.

When describing our findings, we would like to underline the fact that, among the participants of group I, there can be found 3 specific subgroups based on antibody levels (Fig. 1). One consists of participants without knowledge or symptoms of a previous COVID-19 infection but with positive antibody results ($n = 21$; 12.28% of group I, 7.05% of all participants). The 2nd group includes participants with a proven previous infection but with no antibodies found in the first blood sample ($n = 23$; 13.45% of I group, 7.72% of all participants). The 3rd group consists of seropositive convalescents ($n = 127$) with a median age of 45 (40.0–52.0) years, a maximum age of 69 years, and a median number of antibodies of 123.2 (58.7–252.8) IU/mL. In this subgroup, the time between sample collection and symptoms of the infection varied between 18 days and 7 months.

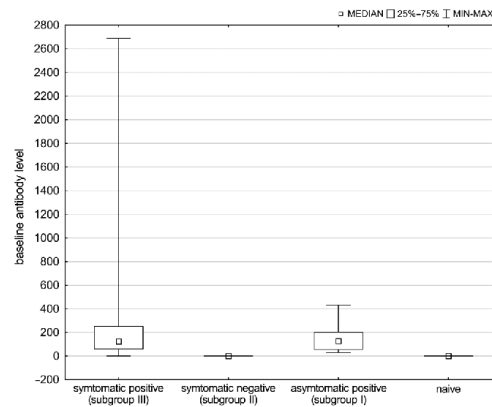


Fig. 1. Initial antibody levels in subgroup I (convalescents, asymptomatic), subgroup II (symptomatic, seronegative) and subgroup III (symptomatic, seropositive)

Subgroup I participants without knowledge of a previous infection were aged from 24 to 50 years, with antibody levels between 28.8 IU/mL and 432 IU/mL (median: 126.4 IU/mL, range: 54.4–2000.0 IU/mL). After receiving the serological results, we confirmed that the participants were not able to recall any possible COVID-19 manifestations.

Subgroup II includes participants with undetectable antibody levels despite the fact that they had a previous SARS-CoV-2 infection and were aged from 23 to 63 years ($n = 23$). The time between COVID-19 and blood sampling varied from 2.5 months to 6.5 months.

Table 2. Characteristics of the subgroups of coronavirus disease (COVID-19) convalescents (group I)

Parameters		Subgroup I (n = 21)	Subgroup II (n = 23)	Subgroup III (n = 127)	Statistical analysis
Age [years]	M ±SD	41.5 ±7.1	40.6 ±9.9	45.0 ±9.5	K-W test H = 6.70, p = 0.0350 I R = 72.690; II R = 66.717; III R = 91.693 I compared to II: Z = 0.399737 I compared to III: Z = 1.629365 II compared to III: Z = 2.226194 I compared to II: p = 1.000000 I compared to III: p = 0.309708 II compared to III: p = 0.078004
	median (IQR)	42.0 (39.0–46.0)	43.0 (33.0–46.0)	45.0 (40.0–52.0)	
Sex, n (%)	female	11 (52.38)	13 (56.52)	77 (60.63)	I compared to II: p = 0.625 I compared to III: p = 0.0983 II compared to III: p = 0.0271
	male	10 (47.62)	10 (43.48)	50 (39.37)	
Previous allergic reactions to vaccines, n (%)	yes	0 (0)	1 (4.35)	2 (1.57)	I compared to II: p = 0.334 I compared to III: p = 0.563 II compared to III: p = 0.382
	no	0 (11.0)	22 (95.65)	125 (98.43)	
Musculoskeletal pain, n (%)	yes	0 (0)	0 (0)	4 (3.15)	I compared to III: p = 0.410 II compared to III: p = 0.388
	no	21 (100)	23 (100)	123 (96.85)	
Fatigue, n (%)	yes	0 (0)	0 (0)	9 (7.09)	I compared to III: p = 0.208 II compared to III: p = 0.188
	no	21 (100)	23 (100)	118 (92.91)	
Taste and smell loss, n (%)	yes	0 (0)	1 (4.35)	15 (11.81)	I compared to II: p = 0.334 I compared to III: p = 0.252 II compared to III: p = 0.557
	no	21 (100)	22 (95.65)	112 (88.19)	
Anti-SARS-CoV-2 antibodies level [IU/mL]	before vaccination M ±SD	145.0 ±112.1	0 ±0	233.0 ±367.7	K-W test H = 6.70, p = 0.0350 I R = 93.286; II R = 12.500; III R = 96.948 I compared to II: Z = 5.470218 I compared to III: Z = 0.317368 II compared to III: Z = 7.606769 I compared to II: p = 0.000000 I compared to III: p = 1.000000 II compared to III: p = 0.000000
	median (IQR)	126.4 (54.4–200.0)	0	123.2 (58.7–252.8)	

subgroup I – asymptomatic; subgroup II – symptomatic, seronegative; subgroup III – symptomatic, seropositive; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; M ±SD – mean ± standard deviation; IQR – interquartile range; I – subgroup I; II – subgroup II; III – subgroup III; K-W test – Kruskal-Wallis test, was used when Shapiro-Wilk test result was $p < 0.05$. For other parameters, the Mann-Whitney U test was used.

Discussion

Assuming that 4 main COVID-19 waves in 2020 and 2021 can be distinguished, the current study covers the 2nd (most convalescent participants suffered from a SARS-CoV-2 infection between October and December 2020) and 3rd wave (when we collected blood samples).

A precise analysis of group I (divided into: subgroup I – convalescents, asymptomatic; subgroup II – symptomatic, seronegative; and subgroup III – seropositive, symptomatic) showed that there was a significant age difference. In subgroup III, the age was significantly higher than in subgroup II (median 45.0 (40.0–52.0) years compared to 43.0 (33.0–46.0) years $p = 0.0271$, Mann-Whitney U test, $U = 1038.5$) and than with group II (45.0 (40.0–52.0) years compared to 43.0 (39.0–47.0) $p = 0.0228$, Mann-Whitney U test, $U = 6675.5$). There was no such age difference between subgroups III and I (45.0 (40.0–52.0) years compared to 42.0 (39.0–46.0) years, $p = 0.0983$, Mann-Whitney U test, $U = 1032.5$). We also analyzed these data using the Scheffé's test (mean square error (MSE) = 57057,

degrees of freedom (df) = 290.00), which showed that, with regard to age, the antibody results differed between subgroup III and group II ($p = 0.00000$), and between subgroups II and III ($p = 0.000452$). These results are consistent with an earlier meta-analysis, which concluded that elderly or older participants (age ≥50 years) are at a higher risk of severe disease course.⁶

Furthermore, the asymptomatic subgroup may be an important link in the transmission of the virus. These participants took part in our study in mid-April–mid-May, so it is possible that they were infected during the so-called 3rd wave. It has been established that individuals without symptoms are capable of infecting others.^{7,8} A similar situation occurs when participants are isolated with a delay due to developing symptoms 1–2 days after becoming infectious.⁹

The level of antibodies in group I differed across individuals, starting from 27.2 IU/mL, which is a borderline titer (blood sample taken 6.5 months after infection), and going up to 2688.2 IU/mL (blood sample taken 2.5 months after infection). The average titer was 190.3 IU/mL. These

differences in titer may be explained by the varying times between SARS-CoV-2 infection and testing. It has been observed that spike antibody levels are relatively stable for several months before waning.¹⁰ In addition, antibody levels are better sustained in participants with severe symptoms.¹¹ Our participants with borderline titers had no or mild symptoms, whereas the participant with the highest result was hospitalized due to the severity of COVID-19.¹² These findings, together with the theory that some people are serological non-responders,¹³ can also partially explain why a subgroup of 23 participants (subgroup II) did not have a detectable level of antibodies.

Limitations

There are several limitations to the current study. First, there are uneven sample sizes due to the presence of asymptomatic convalescents. In addition, there were irregular time intervals between taking blood samples and receiving first vaccine dose.

Conclusions

The symptoms of COVID-19 were more often observed in older participants, and the severity of the disease can correlate with higher antibody titers seen later after COVID-19 compared to a mild infection. One should be aware that numerous participants without symptoms of past SARS-CoV-2 infection can transmit the virus to other people. Serological data are not an unambiguous evidence of a past infection.

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Article

How Humoral Response and Side Effects Depend on the Type of Vaccine and Past SARS-CoV-2 Infection

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Abstract: Since the end of December 2020, it has been possible to vaccinate against COVID-19. Our aim was to evaluate and compare the effectiveness of the vaccines available at the time of the mass vaccination program in Poland and also to look into the most common adverse side effects. Patients' anti-SARS-CoV-2 antibodies levels were checked before vaccination and after the first and after the second/last dose by the anti-SARS-CoV-2 QuantiVac ELISA (IgG) (EUROIMMUN Medizinische Labordiagnostika AG; Luebeck; Germany) test. Before each blood collection, all patients filled out a questionnaire regarding experienced side effects. We observed that 100% of patients responded to the vaccinations. After the first dose, convalescents had much higher levels of anti-SARS-CoV-2 antibodies than naive patients, although after the second dose, 61 out of 162 convalescents (37.7%) had lower results than before. The comparison of immunological responses in the convalescents group after the first dose and in the naive group after the second dose showed that convalescents had higher antibody titers, which may suggest the possibility of changing the vaccination schedule for convalescents. The highest antibody titers after both the first and second doses were observed after Moderna shots. Fever was identified as a significant factor regarding higher levels of antibodies after the first and second doses of the vaccine.

Keywords: SARS-CoV-2 vaccines; COVID-19; humoral immune response; adverse effects of vaccines; vaccination program

1. Introduction

Since the end of December 2020, it has been possible to start vaccination against COVID-19. Safe and effective vaccines were introduced at least one year after the beginning of the COVID-19 pandemic and have helped to control it in many regions of the world. There are five vaccines used in Poland which have been approved for use by EMA. Two of them are mRNA vaccines—Pfizer/BioNTech and Moderna (New York, NY, USA)—and the other two are based on the adenoviral vectors by AstraZeneca and Johnson&Johnson and the last one, the recently introduced Nuvaxovid (Novavax), is a protein subunit vaccine.

Many studies have started to evaluate the efficacy, safety and tolerability of the different types of vaccines. It turns out that COVID-19 vaccines provide sufficient protection against serious illness and death, although the efficacy can be lower in terms of new variants such as Delta and Omicron—especially sub-lineages BA.4 and BA.5, which are now

classified as VOC [1]. On the other hand, vaccination can prevent to some extent transmission of the virus to others. However, waning of the immune response and appearance of ‘breakthrough infections’ in fully vaccinated patients can be seen. It can happen more often in older people with underlying medical conditions or who have high-risk exposures to SARS-CoV-2 [2,3].

It has been established that additional doses of COVID-19 mRNA (Pfizer or Moderna) vaccines should be used to improve decreased protection after the primary vaccination schedule, especially in terms of new VOCs. The CDC has approved booster doses: a first booster dose for everyone 5 years of age and older, and the second booster for adults 50 years of age and older and persons 12 years of age and older who are immunocompromised [4].

Still, there are not enough data on the size and duration of natural immunity and immunity induced by vaccination. It can depend on viral, host and demographic factors. There are reports indicating that convalescents achieve enough protection soon after one vaccine dose and that antibody titers after the first dose in convalescents and the second dose in patients with no previous infection are similar. However, data concerning long-term protective immunity in both convalescents and naïve patients require more studies [5–8].

Many individuals experienced side effects due to vaccination. Most often, they are mild to moderate and include fever, headache, fatigue, malaise, myalgia, chills, pain or redness at the injection site. Sometimes, side effects are more serious. Unexpected side effects of COVID-19 vaccines such as allergic reactions, anaphylaxis, immune thrombocytopenia, cerebral sinus venous thrombosis or splanchnic vein thrombosis with antibodies to platelet factor 4 have been reported [9].

In mRNA COVID-19 vaccination, rare cases of myocarditis and pericarditis, mostly among males ages 12 through 39 years, have been reported [10,11].

Seroconversion panels based on serial tests of blood samples collected before and after each vaccine dose can demonstrate useful methods to control exposure to SARS-CoV-2 and vaccine effectiveness.

The aim of this study was to evaluate anti-SARS-CoV-2 antibody response after the first and the second doses of vaccines in a cohort of adult patients (previously infected or not infected) from the Lower Silesia Region, Poland, and to compare antibody titers after each vaccine dose in these two groups. We also looked for the most common adverse effects between different types of COVID-19 vaccines and possible SARS-CoV-2 infections during and after vaccinations.

2. Materials and Methods

The study received approval of the Bioethic Committee at Wrocław Medical University (No 51/2021).

2.1. Study Group

The single-center study was performed at the Medical University of Wrocław, Poland, between 20 February 2021 and 25 August 2021. The patients were recruited by an announcement in local media such as newspapers, television and the local hospital’s website. They filled in a contact form and were called or messaged by members of our team to confirm their willingness to be vaccinated against SARS-CoV-2, to take part in our study and to initially exclude any contraindications. Later, in person, the volunteers signed a questionnaire, where they had to provide information about exclusive diseases such as diabetes, any cancer within the last 5 years, chronic kidney, liver or lung diseases, AIDS or immunosuppression for any other reason. The patients registered for a specific date. The choice of the vaccine and the facility providing COVID-19 shots were random. One of the vaccines against COVID-19 registered at that time in the EU (Pfizer, Moderna (Cambridge, MA, USA), Astra Zeneca (Cambridge, UK) or Johnson & Johnson (New Brunswick, NJ, USA)) was subsequently used. Each patient was informed about the aim of the study. Patients were informed that they could withdraw their consent at any stage of the study.

and they signed informed consent. It was also necessary for the volunteers to sign in for the vaccination against SARS-CoV-2 before joining the study. In the first part of the study, we assessed anti-SARS-CoV-2 antibody levels before vaccination. Due to frequent changes in registration rules and vaccination dates and patients' individual health contraindications at the moment, the interval between collecting blood samples for testing and the first dose of vaccine varied from 1 day to 6 weeks, usually approximately 1 week.

Later, we invited the patients for antibody level follow-ups after the first dose, precisely up to 7 days before the second vaccination and again 4–5 weeks after the second/last dose.

Inclusion criteria: Patients over 18 years of age who signed informed written consent to participate in the study and were willing to be vaccinated were included. The patients disclosed whether they passed SARS-CoV-2 infection and if it was confirmed with PCR or serological test.

Exclusion criteria: Those who suffered from diabetes, any cancer within the last 5 years, chronic kidney, liver or lung diseases, AIDS or immunosuppression for any other reason were excluded.

Before each blood sample was taken, at every visit, information on adverse events related to vaccination and general health conditions was collected. Questions in a self-administered questionnaire concerned general well-being, persistence of COVID-19 symptoms, adverse vaccine reactions, treatment administered, chronic diseases and allergic reactions to drugs, substances and foods. Patients were helped to complete the questionnaire by a team consisting of a doctor and a nurse.

According to the inclusion and exclusion criteria and questionnaire data, 298 patients, citizens of Lower Silesia region, Poland, mostly from Wrocław, aged 21–69 of both sexes were enrolled to the study. Originally, both groups had similar sizes, but 21 patients declaring themselves as naive, with no history of COVID-19 symptoms or laboratory confirmation of the infection, had antibodies present before vaccination. This subgroup of asymptomatic convalescents was separated from the naive group and transferred to the convalescents group. Based on final serological results obtained before vaccination in the whole study group, two groups of patients were established:

- Group I of 171 COVID-19-convalescent individuals with positive results on PCR, antigen or serological tests confirming the presence of IgG antibodies or other strong indication of past infection (e.g., loss of sense of smell after living with someone with confirmed infection), within 6 months prior to qualification for this study;
- Group II of 127 patients without evidence of previous SARS-CoV-2 infection and with 0 anti-SARS-CoV-2 antibodies before vaccination (naive patients).

During the study, the numbers of each group had been changing due to COVID-19 diagnoses after the first dose of vaccination, resigning from the study after the first or second test, admitting volunteers after the first dose if they presented individual IgG antibody results from before the shot. These factors resulted in the following number of participants tested in each phase of the study:

D0 (test before vaccination) = 298 participants (group I: 171, group II: 127);

D1 (test after 1st dose) = 286 (I: 163, II: 123);

D2 (test after 2nd/last dose) = 295 (I: 169, II: 126).

All individuals participated in the first part of the study, in which we assessed initial antibody titers (before vaccination). The manuscript titled Epidemiological and retrospective study in cohort qualified for anti-SARS-CoV-2 vaccination in the region of Lower Silesia, Poland was sent to the Editor of a journal for publication. The majority of the study group received mRNA vaccination (Pfizer $n = 191$; Moderna $n = 70$), while others chose vector shots (Astra Zeneca $n = 25$, Johnson&Johnson $n = 9$). Since Johnson&Johnson full vaccination consists of only one dose, individuals vaccinated with it were tested just once and their results were compared with the results of the other patients after the second doses of other vaccines.

Patients were obliged to come for a visit before the first (D0) and the second dose of vaccine (D1) (up to 7 days before the second dose) and also 4–5 weeks after the second /last

dose (D2). The intervals between D0 and D1 varied greatly due to different time periods between the first and second vaccines (originally, for Pfizer 21 days, Moderna 28 days, Astra Zeneca 3 months, but the recommendations changed with time).

2.2. The Procedure

Blood samples were taken before (D0) the first and second dose of vaccine (D1) (up to 7 days before second dose) and 4–5 weeks after the second/last dose (D2).

At each visit, the patients were tested for anti-SARS-CoV-2 IgG antibodies. Plasma samples were collected using heparin, centrifuged and stored in aliquots at -70°C for later use. The anti-SARS-CoV-2 QuantiVac ELISA (IgG) (EUROIMMUN Medizinische Labordiagnostica AG, Luebeck, Germany) was used for quantitative detection of anti-SARS-CoV-2 antibodies by means of a 6-point calibration curve.

In the quantitative enzyme-linked immunoabsorbant assay, the S1 domain of the spike protein of SARS-CoV-2 including the receptor binding domain (RBC) was used as an antigen. ELISA assay was performed and the results were evaluated as recommended by the manufacturer. Samples with absorbance higher than the absorbance of the highest standard (386 IU/mL) were diluted and retested. The final results were calculated by multiplication by a dilution factor. The assay was standardized against “First WHO International Standard for anti-SARS-CoV-2 immunoglobulin” (NIBSC 20/136), so the quantitative results are given in standardized units: IU/mL (IU—international units) which are identical to BAU/mL (BAU—binding antibody units).

2.3. Statistical Analysis

For each parameter, mean, median (M), standard deviation (SD), range (min, max), lower and upper quartile (25Q, 75Q) were calculated. Statistical significance between means for independent groups was calculated by one-way analysis of variance (ANOVA), alternatively using the non-parametrical Mann–Whitney U test, when the variances in groups were heterogeneous (the homogeneity of variance was determined by the Levene’s test).

Statistical significance between frequencies was calculated by the chi-square test with corresponding degree of freedom df ($df = (m - 1) * (n - 1)$, where m —number of rows, n —number of columns). A p value of less than 0.05 was required to reject the null hypothesis. Statistical analysis was performed using EPIINFO Ver. 7.2.4.0 and Statistica Ver. 13.3. software packages.

The primary outcome was antibody response to 4 different vaccines at D1 and D2 in two groups of patients: convalescents and those with no evidence of previous SARS-CoV-2 infection. The secondary outcome was the frequency and the type of adverse effects following the first (D1) and the second (D2) dose of vaccine reported in the questionnaire.

3. Results

At the beginning of the project, 298 participants were divided into two groups—group I: 171 patients with previous SARS-CoV-2 infection (in the initial tests before vaccination, the median results for men was 164.7 IU/mL, for women 226.6 IU/mL) and group II: 127 naive patients (initial tests did not detect any antibodies). Table 1 presents the characteristics of the group of patients. This table in a modified version was also presented in the previous paper sent to another journal and is currently under review.

In this paper, we were focusing on participants that took part in second (D1) and third (D2) antibody tests. Therefore, total abundance on each step of the study slightly differs.

From group I, 150 patients had test-proven or highly possible SARS-CoV-2 infection between 1 October 2020 and 5 April 2021, of which 3 were hospitalized due to the severity of COVID-19 symptoms. Additionally, 21 people were found seropositive without any knowledge of previous infection.

Table 1. Characteristics of the groups.

Parameters		Group I (n = 171)	Group II (n = 127)	p Value
Age	Mean $\pm$ SD	44.0 $\pm$ 9.4	42.6 $\pm$ 7.2	0.177
	Me [IQR]	44.0 [39.0–49.0]	43.0 [39.0–47.0]	
Sex	female	101 (57.81%)	74 (58.27%)	0.828
	male	70 (41.19%)	53 (41.73%)	
Anti-SARS-CoV-2 antibodies level [IU/mL]	Before vaccination (Mean $\pm$ SD)	190.3 $\pm$ 328.4	0	<0.001
	Me [IQR]	105.6 [38.4–198.4]	0	
SARS-CoV-2 infection in the past	yes	150	0	
	no	21	127	
Vaccine type for D2	Pfizer	104	87	
	Moderna	37	33	
	Astra Zeneca	21	4	
	Johnson&Johnson	6	3	

SD—standard deviation; Me—median; IQR—interquartile range.

The mean time between the diagnosis and 1st dose of vaccine totals 153.7 days, median 169 [IQR 128.0; 185.0].

The mean age of both groups was similar and there were no significant differences in terms of sex, with slight predominance of women in the groups. There were no differences between group I and group II regarding co-morbidities.

At the baseline, anti-SARS-CoV-2 IgG antibodies were found only in convalescent group I with a median number of 105.6 [38.4–198.4] IU/mL. Results obtained in men and women did not differ. In group II, no spike-antibodies were seen (0 IU/mL).

All participants received a COVID-19 vaccine (Pfizer-BioNTech—64.74% or Moderna 23.73%, AstraZeneca (Cambridge, UK) 8.47%, Johnson&Johnson 3.05%) and were tested up to 7 days before the second dose of two-dose vaccines and 4–5 weeks after the second (or last dose in those receiving the Johnson&Johnson vaccine). During this part of the study, mixing vaccines was not allowed, meaning the second shot had to be the same as the first one.

From all participants, we received information about only one SARS-CoV-2 infection after the first dose of the Moderna vaccine. This woman was vaccinated on 23 April 2021, and she received a positive RT-PCR result on 5 May 2021. The second dose was administered on 21 June 2021. Before vaccination, she was not infected and her initial antibody level from a sample taken on 21 April 2021 was 0 IU/mL. After infection, she did not match any of the two analyzed groups; therefore, she was excluded from further analysis.

Levels of anti-SARS-CoV-2 antibodies and increase of antibody levels were analyzed separately in each group and also compared between group I and group II after each vaccine dose.

3.1. Group I—Convalescents

In group I, 86.5% were initially seropositive (before vaccination). After the first dose (0–7 days before 2nd dose), 98.77% of 163 participants had IgG antibodies present in the blood samples; 2 people did not respond to the vaccine:

- patient 1 (group I)—male, age 27, symptomatic SARS-CoV-2 infection in the past, antibodies levels: D0 = 57.6; D1 = 0; D2 = 2860 IU/mL
- patient 2 (group I)—female, age 39, symptomatic SARS-CoV-2 infection in the past, antibodies levels: D0 = 0; D1 = 0; D2 = 1318 IU/mL

In group I, a positive correlation was found between baseline (D0) and D1 antibody levels ($p = 0.0352$). The results after the first dose are presented in Table 2.

Table 2. Antibody levels after the first dose of anti-SARS-CoV-2 vaccine in convalescents [IU/mL].

Group	Mean	N	SD	MIN	MAX	Me [IQR]
BASELINE = 0	3684.8	23	2423.4	0.0	7936.0	3699.2 [1248.0; 5239.5]
BASELINE > 0	5826.5	138	4586.1	0.0	25,600.0	4862.3 [2944.0; 6336.0]

SD—standard deviation; Me—median; IQR—interquartile range.

We did not find a similar correlation between baseline and D2 antibody levels ($p = 0.135$).

3.2. Group II—Naïve Patients

In group II, no anti-SARS-CoV-2 antibodies were present initially. After receiving the first vaccine dose, 98.37% of patients responded; two patients did not produce antibodies:

- patient 3 (group II)—male, age 61, antibodies levels: D0 = 0; D1 = 0; D2 = 1050 IU/mL
- patient 4 (group II)—female, age 38, antibodies levels: D0 = 0; D1 = 0; D2 = 2464 IU/mL

In general, in both groups we observed a considerable increase of antibody levels after the first dose; in group I, there was a fifty-two-fold increase. Antibody levels were significantly higher titers after the first dose (D1) in group I compared to values obtained in group II (median group I: 5501.5; group II: 751.9 IU/mL) and after the second dose (D2) (median: group I: 5523.7; group II: 3625.6 IU/mL). For detailed information, see Table 3.

Table 3. Anti-SARS-CoV-2 IgG antibody levels at each stage of the study.

	Anti-SARS-CoV-2 IgG Antibodies Levels [IU/mL]					
	D0—Baseline		D1—After 1st Dose		D2—After 2nd/Last Dose	
	Mean ± SD	Me [IQR]	Mean ± SD	Me [IQR]	Mean ± SD	Me [IQR]
Group I	190.3 ± 328.4	105.6 [38.4; 198.4]	5501.5 ± 4380.0	4736.0 [2676.9; 6144.0]	5523.7 ± 4016.4	4560.0 [3040.0; 7232.0]
Group II	0.00 ± 0.00	0.0 [0.0; 0.0]	751.9 ± 897.7	451.2 [217.6; 915.2]	3625.6 ± 2568.9	3056.0 [2048.0; 4512.0]
<i>p</i> -value	0.00000		0.00000		0.00000	

SD—standard deviation; Me—median; IQR—interquartile range.

3.3. Lower Results after Second Dose of Vaccine

After the second dose, all participants reacted in an expected way and in 100%, samples taken 4–5 weeks after the full vaccine scheme, IgG antibodies against SARS-CoV-2 were found (including 4 people who did not respond to the first dose). In group I, we observed a slight increase in mean antibody titers (5501.5 vs. 5523.7 IU/mL), whereas in group II the increase was visibly higher (751.9 vs. 3625.6 IU/mL, which is an almost fivefold increase).

These differences in responding to the first and second doses in each group (group I presented a minimal increase in antibodies between D1 and D2, whereas group II presented continuous growth of antibody levels) led us to look deeper into the collected data. We were able to separate out from group I (convalescents) patients who had lower levels of antibodies after the second dose than after the first dose—61 out of 162 analyzed individuals (37.7%). We distinguished this subgroup with the letter 'A' and rest of the patients who had as-expected higher antibody titers after second dose with the letter 'B'. A careful analysis of this topic showed that there was a positive correlation between a higher amount of antibodies after the first dose and the possibility of having a lower result after the second dose—the higher the antibody levels after the first dose, the more likely they would fall despite/after the second dose ($p = 0.0001$). We also discovered a possible influence of age on increased antibody production: younger participants were in subgroup (A) with lowering antibody results after the second dose (mean age 42.3 vs. 45.4; $p = 0.0418$). We did

not observe a correlation between the analyzed changes and baseline antibody levels ($p = 0.939$) or results after the second dose ($p = 0.0542$). For more, see Table 4.

Table 4. Comparison of two subgroups from group I—patients who presented lower antibodies level after second dose of vaccine (A) vs. patients who presented constant increase of antibodies level (B).

	Group I	Mean	N	SD	MIN	MAX	Me [IQR]
Age ($p = 0.0418$) [years]	A	42.3	61	9.7	21.0	68.0	43.0 [38.0; 46.0]
	B	45.4	101	9.2	24.0	69.0	45.0 [40.0; 52.0]
Baseline level ($p = 0.939$) [IU/mL]	A	189.4	61	287.0	0.0	1600.0	120.0 [46.4; 188.8]
	B	185.4	99	338.2	0.0	2688.2	83.2 [38.3; 204.8]
after 1st dose ($p = 0.0001$) [IU/mL]	A	6983.1	61	4698.9	787.2	21,800.0	5376.0 [4000.0; 8256.0]
	B	4606.9	101	3960.2	0.0	25,600.0	3840.0 [2080.0; 5587.2]
After 2nd dose (0.0542) [IU/mL]	A	4811.3	61	3192.5	533.0	19,840.0	3968.0 [3008.0; 6048.0]
	B	4879.7	101	4397.9	486.4	28,200.0	4992.0 [3132.0; 7936.0]

SD—standard deviation; Me—median; IQR—interquartile range.

3.4. Antibody Titers after First Dose in Convalescents and after Second Dose in Naïve Patients

Another interesting observation concerns the antibody levels in group I after the first dose and group II after the second dose. Aware of the fact that previous SARS-CoV-2 infection is similar to immune priming and the first vaccine dose in group I can be compared to a booster, we analyzed antibody levels reached after the first dose of the vaccine in convalescent group I and after the second dose in naïve group II.

Assuming antibody levels are a sufficient measurement method of immunological response, it may be concluded that convalescents had been more immunologically stimulated with one dose than naïve patients even with two doses ($p = 0.00003$). In comparing group I after the first dose vs. group II after the second dose, the numbers were as presented: mean $\pm$ SD 5501.5 $\pm$ 4380.0 vs. 3625.6 $\pm$ 2568.9 IU/mL; M [IQR] 4736.0 [2676.9; 6144.0] vs. 3056.0 [2048.0; 4512.0].

3.5. Specific Vaccines

During the analysis of the collected data, a summary of antibody results after particular vaccines was prepared (see Table 5). Because of the previously explained factors, the number of participants at each stage of the study was slightly different. The group sizes were as follows: for Astra Zeneca D0 = 24, D1 = D2 = 25; Johnson&Johnson D0 = D1 = D2 = 9; Moderna D0 = D1 = D2 = 70; Pfizer D0 = D1 = 192, D2 = 191. The highest antibody titers after both the first and second doses were observed after Moderna shots.

Table 5. Antibodies levels after particular SARS-CoV-2 vaccines.

	Astra Zeneca		Johnson&Johnson		Moderna		Pfizer	
Antibodies level	Mean $\pm$ SD	Me [IQR]	Mean $\pm$ SD	Me [IQR]	Mean $\pm$ SD	Me [IQR]	Mean $\pm$ SD	Me [IQR]
baseline	283.5 $\pm$ 608.3	88.0 [33.6; 176.8]	219.4 $\pm$ 502.1	28.8 [0.0; 134.4]	101.9 $\pm$ 245.1	0.0 [0.0; 96.0]	84.7 $\pm$ 163.5	0.0 [0.0; 122.4]
After 1st dose	1800.0 $\pm$ 1611.1	1376.0 [787.2; 2768.3]			4851.6 $\pm$ 5236.0	3232.0 [1014.4; 6080.0]	3228.3 $\pm$ 3777.9	2256.0 [358.4; 5024.0]
After 2nd/last dose	2841.8 $\pm$ 2398.6	1920.0 [1074.0; 3904.0]	1337.4 $\pm$ 1448.6	844.8 [140.8; 1920.0]	4733.1 $\pm$ 4733.1	5316.0 [3904.0; 8540.0]	4369.1 $\pm$ 2893.1	3360.0 [2464.0; 5456.0]

SD—standard deviation; Me—median; IQR—interquartile range.

None of the participants was infected with SARS-CoV-2 4 weeks after full vaccination.

Table 6 shows the relationship between the effect of the second dose of the vaccine depending on the formulation administered. We observe a statistically significant difference between the Astra Zeneca, Moderna and Pfizer preparations.

Table 6. Antibodies levels after particular SARS-CoV-2 vaccines.

	Astra Zeneca	Johnson& Johnson	Moderna	Pfizer
Astra Zeneca	-	-	-	-
Johnson& Johnson	U = 80.0 <i>p</i> -value = 0.1675	-	-	-
Moderna	U = 547.5 <i>p</i> -value = 0.0062	U = 250.0 <i>p</i> -value = 0.1236	-	-
Pfizer	U = 1592.5 <i>p</i> -value = 0.0135	U = 780.5 <i>p</i> -value = 0.3049	U = 6024.0 <i>p</i> -value = 0.0342	-

SD—standard deviation; Me—median; IQR—interquartile range.

The below Figure 1 shows that Moderna induces the production of antibodies the most, similarly to Pfizer, but Astra Zeneca and Johnson&Johnson are the least active.

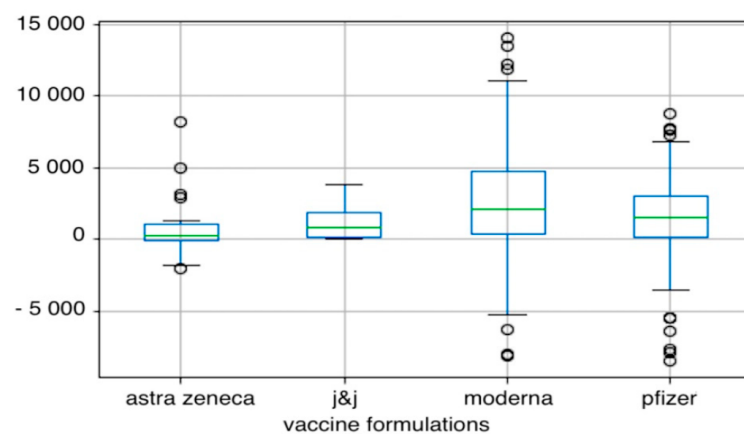


Figure 1. The figure shows the difference in antibody levels after the administration of the second dose of the individual vaccine formulations [IU/mL]. J&J—Johnson&Johnson. The main body of the boxplot shows the quartiles, horizontal lines in the middle of each box are medians, whiskers is the vertical lines extending to the most extreme, non-outlier data points and fliers represent data that extend beyond the whiskers.

3.6. Adverse Effects

During the study, the adverse events after vaccinations were strictly reported. Most of them were mild symptoms lasting 1 or 2 days. We analyzed them separately regarding groups I and II and also first and second doses of vaccines. Reported side effects are presented in Figures 2 and 3.

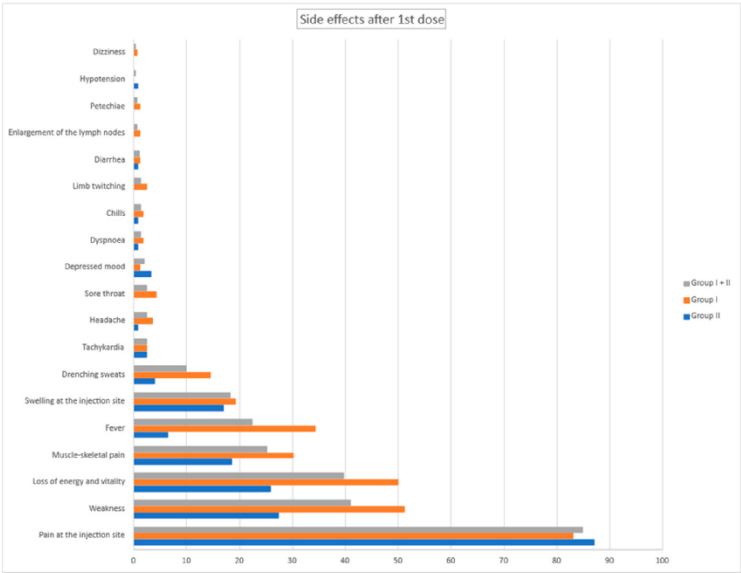


Figure 2. Side effects after 1st dose for group I and group II in %.

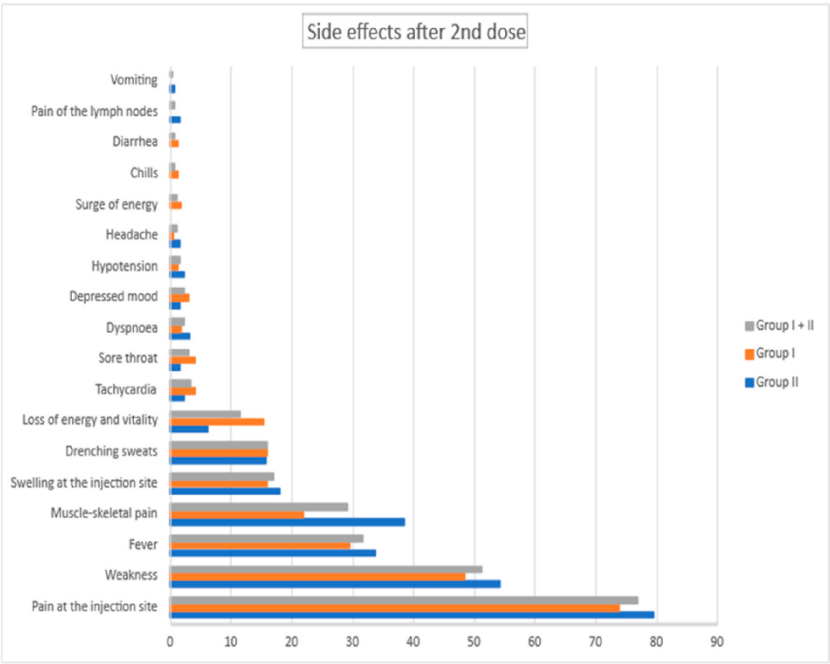


Figure 3. Side effects after 2nd dose for group I and group II in %.

Some of the symptoms turned out to be more significant in relation to antibody production. In group I, we were able to identify increased temperature as a particular factor. We found a correlation between the presence of fever after the first dose with a higher antibody titer baseline ($p = 0.0476$), after the first dose ($p = 0.0222$) and second dose ($p = 0.00026$), and this was the only significant symptom after the first dose. Fever after the second dose was related to higher antibody levels after the first dose ($p = 0.00027$) and second dose ($p = 0.00002$). Weakness after the second dose was visibly associated with the immunological response after the first dose ($p = 0.00000$) and second dose ($p = 0.00000$). There is also a possible correlation between pain at the injection site after the second dose and antibody levels after the first dose ($p = 0.0458$) and second dose ($p = 0.0161$) and between muscle and joint pain after the second dose with antibody results after the first dose ($p = 0.00613$) and second dose ($p = 0.0221$).

Similar correlations were observed in group II regarding symptoms after the second dose: between fever and antibody titers after the first dose ($p = 0.00001$) and second dose ($p = 0.00026$); between pain at the injection site and antibody levels after the second dose ($p = 0.00515$); between weakness levels after the first ($p = 0.0107$) and second dose ($p = 0.0114$); and between muscle and joint pain and antibody titers after the first ($p = 0.00815$) and second dose ($p = 0.0460$).

We also arranged the adverse events with regard to specific vaccine types. The data are presented in Figures 4 and 5.

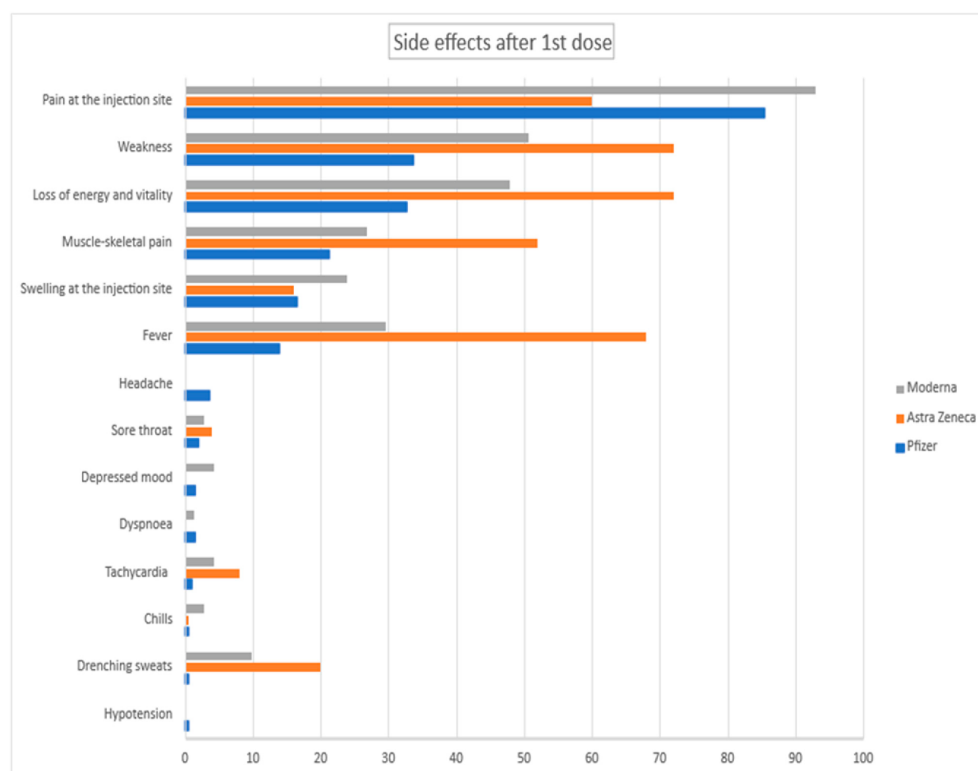


Figure 4. Side effects after 1st dose for specific vaccines in %.

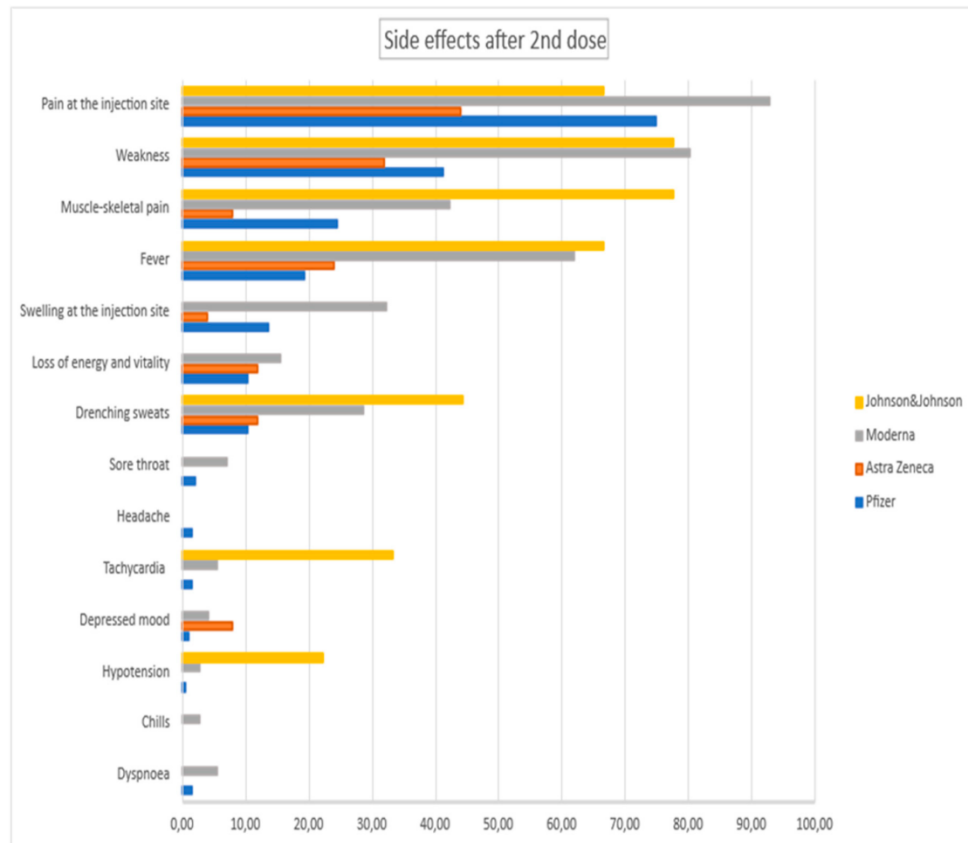


Figure 5. Side effects after 2nd dose for specific vaccines in %.

4. Discussion

We reported the antibody response (IgG) after the first and the second doses of four different vaccines against COVID-19 authorized by the FDA and EMA in a cohort of adult patients from the Lower Silesia region in Poland reflecting the general population. These patients were divided into two groups: group I with previous SARS-CoV-2 infection and group II not infected before vaccination. Analysis of different parameters such as age, sex, time elapsed since natural infection that could influence humoral response and adverse side effects were explored. Although immunological defense is based on many different mechanisms, we believe that analyzing precise antibody titers, as a simple, minimally invasive, cheap and widely available method contributes strongly to the validation of the effectiveness of the COVID-19 vaccines. We compared the results between group I and group II in relation to their baseline antibody levels as well as antibody levels after the first dose in group I and second dose in group II. We also analyzed the group of patients with previous infection in whom antibody levels after the second dose were lower than after the first dose of vaccine.

We found that the presence of antibodies at baseline influenced the response to the vaccine after the first dose, but not after the second one. In general, previous SARS-CoV-2 infection resulted in significantly higher IgG anti-SARS-CoV-2 antibody levels compared

with group II without evidence of previous infection. Our results are consistent with the data reported by others. B. Wolszczak-Biedrzycka reports that 8 months after two doses of the Pfizer vaccine among health workers, anti-SARS-CoV-2 S antibodies were still detectable and considerably higher in the group of convalescents compared to people vaccinated without a history of SARS-CoV-2 infection [12].

Regarding our results, Mendoza-Gonzales et al. found that antibody concentrations after the second dose of Pfizer-BioNTech were similar in both groups although significantly higher among convalescents after the first dose [13]. In our study, convalescents also presented higher antibody titers after the second dose. Paul R. Wratil et al. reported that convalescents developed a higher neutralization capacity against all SARS-CoV-2 variants of concern than naive individuals after vaccination, and that in naive individuals, the infection-neutralization capacity after the second vaccination was significantly lower than that of vaccinated convalescents [14].

It was observed that previous infection may be compared to the first vaccine dose because of the immune priming, and the first dose of vaccine would be similar to the booster. There is a question of whether the second dose in a basic vaccination schedule of an anti-COVID-19 vaccine in the group of patients previously infected is needed. There are a few reports indicating that previously acquired immunity due to infection with SARS-CoV-2 is connected to the level of antibody response to the first vaccine dose similar to that achieved in naive individuals after the second dose [8,15,16].

The results published by Jung et al. and Gaebler et al. show that individuals with past SARS-CoV-2 infection produce memory B and T cells that protect against re-infection even for 10 months [17,18]. Additionally, antibodies can be detected even 10 months after infection in unvaccinated individuals [13]. This may be the basis for creating future recommendations regarding vaccination of convalescents. The decision about the number of doses of the primary vaccination may depend either on the history of previous infection confirmed by positive PCR or antibody tests or antibody levels before the first vaccine dose. Before changing the current vaccination rules, this topic requires further investigation.

This study was conducted while Alpha, Beta and Delta variants were circulating and causing most infections. There are some scientific reports about convalescents benefiting from just one booster shot in terms of protection against Wuhan D614G, Delta and Omicron [19]. These findings are based on an in vitro study of 66 convalescents; therefore, the topic requires further exploration and real-world case examination.

There are no data about the effectiveness and the duration of vaccine-induced antibody responses among previously seropositive and seronegative individuals.

There is not yet information regarding how long after infection one shot would be sufficient. It is probably the same as in current recommendations concerning booster shots for everyone who received the primary series.

Nevertheless, the level of protection in convalescents may be different and dependent on the severity of previous SARS-CoV-2 infection [20,21]. Therefore, mass vaccination would be safer, and giving everyone the full primary series followed by a booster dose to avoid insufficient protection and appearance of viral variants was a simple solution to the emerging health crisis [22].

However, in some countries, results indicating high anti-SARS-CoV-2 antibody levels in convalescents led to temporary changes in the vaccination schedule and the use of a single dose of two-dose vaccine in this group of patients.

Another fact worth focusing on is that 61 out of 162 patients from group I presented lowering antibody results after the second dose compared to the first dose. This phenomenon may be explained by a few factors [23]. First of all, the timing of the sample (4–5 weeks after 2nd dose) may not be optimum for showing increased antibody production. Furthermore, all discussed patients presented substantially higher antibody titers after the first dose, and these titers could block produced SARS-CoV-2 antigens and could weaken the effectiveness of antigen-presenting cells, which would result in a limited immunological response after the second dose. Marie I. Savanovic suggested that this may

also be a reaction to polyethylene glycol used by Pfizer, but in our study only 39 patients received the Pfizer vaccine (16 Moderna and 6 Astra Zeneca). Despite lower antibody levels, the results observed after second doses can still be considered high.

Although there is not much information about lower antibody levels after the second dose compared to the first dose, there are plenty of data considering prolonging intervals between two doses [24]. This shall result not only in a stronger immunological response of immunocompetent individuals, but it may also lower the number of severe adverse events, such as heart inflammation seen in some young men [25]. A total of 37.7% of convalescents did not benefit from the second dose, and an extended interval between two doses can help reduce severe side effects, which is an important factor in the discussion, especially with people skeptical about vaccinations. The individualization of vaccination schemes for convalescents in worldwide terms is possible just like in cases of immunocompromised patients who now receive three doses in the primary series and could be received with understanding and supporting by the general public.

Among our participants, 100% developed antibodies after the second/last dose, but four of them did not respond to the first dose (1.4% from 286 individuals analyzed after the first dose). These findings are consistent with the data obtained in other studies. Gareth Iacobucci reported that 96.42% out of 8517 patients in England and Wales developed antibodies 28 to 34 days after their first dose [26].

All types of vaccines studied in this project led to high antibody titers after a full vaccination schedule. Moderna showed up as the most effective in terms of highest antibody levels, although both mRNA vaccines are proven to be similar in their results [27]. Our findings regarding mild side effects most commonly appearing after Moderna shots are consistent with the available data [28]. This is further proof that mRNA technology is a significant and useful achievement of modern science. It is also worth mentioning that the Moderna vaccine is easier to transport and store than the one produced by Pfizer. Although patients in our study received four types of vaccines, we do not want to state similar conclusions about Astra Zeneca and Johnson&Johnson vaccines since sample sizes in these cases were small.

In the course of analyzing the acquired data, a significant correlation was found between some side effects and higher antibody levels after each dose, such as fever, weakness, pain at the injection site and muscle and joint pain. From the symptoms mentioned above, only fever in convalescents was observed to be relevant after the first dose. For both group I and II, a positive correlation was found between these symptoms reported after the second dose and the antibody titers. William Schaffner, M.D., stated in an interview for Medical News Today that there is no direct correlation between side effects and protection [29]. This does not mean that this stands in contradiction with our findings that focus on antibody levels rather than on protection levels against infection.

4.1. Limitations

We assessed only the humoral immune response in terms of IgG against SARS-CoV-2, aware that it is only a part of the immune response, but very easy to test and cheap. Based on the producer's information, the test can to some extent detect neutralizing antibodies. The convalescent and naïve patient groups consisted of different numbers of patients, and only a few participants chose the Johnson&Johnson vaccine, which made it difficult to compare with other vaccines.

4.2. Conclusions

All four types of vaccines were effective, and they induced serological responses in immunocompetent patients, both in convalescents and naïve patients. The best results came from the Moderna vaccine followed by Pfizer, but both mRNAs worked as expected.

We observed that in the case of naïve patients, antibody levels increased after every dose. In the convalescents group with baseline level > 0 IU/mL, the increase of antibody

levels after the first dose was significantly higher, but the baseline level had no significance regarding results after the second dose.

We compared results obtained from the convalescents group after the first dose and naive group after the second dose, and we conclude that the convalescents had been better immunologically stimulated.

A total of 37.7% of convalescents showed a decrease of antibody titers after the second dose compared to the first dose. It is worth emphasizing that in this subgroup, we had patients receiving Pfizer, Moderna and Astra Zeneca vaccines; therefore, the phenomenon was not dependent on the manufacturer. These are important discoveries which should lead to changes in the prime series of SARS-CoV-2 vaccinations for recent convalescents, especially young people with high antibody levels (e.g., >2080 IU/mL, which is a standard maximum level shown by commercial laboratories). They can benefit from prolonging the intervals between first and second doses in terms of immunological responses and additionally by lowering the risk of rare but severe side effects. We encourage elderly people and those with low antibody levels after past infections not to hesitate and to receive both vaccine doses in the recommended time.

Among the most commonly reported side effects, we found pain at the injection site, weakness, fever and muscle and skeletal pain. In the naive group, the symptoms were more intensified after the second dose, whereas in the convalescents group, they were often observed after the first dose. People receiving the Moderna vaccine more often reported adverse effects in the questionnaire and they also presented higher antibody levels. We are not keen to make similar conclusions regarding Astra Zeneca and Johnson&Johnson vaccines since the groups of patients receiving vector vaccines in our study were relatively small.

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

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

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How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2

Original Study

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Abstract

Introduction. Despite vaccinations available worldwide, patients and healthcare workers still struggle with COVID-19. Previously we observed in some convalescents surprisingly lower antibody levels after a second dose of SARS-CoV-2 vaccine compared to the first dose. Six months after full vaccination we obtained follow-up results of 87 patients divided into 3 groups: (A) convalescents with higher antibody levels after second dose of vaccine compared to first dose, (B) naïve patients, (C) convalescents with decreasing antibody level after second dose compared to first dose.

Materials and Methods. Patients' anti-SARS-CoV-2 antibody levels were checked by the anti-SARS-CoV-2 QuantiVac ELISA (IgG) (EUROIMMUN Medizinische Labordiagnostika AG, Luebeck, Germany) test, as before, and prior to the blood sampling patients completed a questionnaire regarding, inter alia, general condition, smoking, flu vaccination.

Results. Thanks to this follow-up we concluded that none of the patients suffered from symptomatic SARS-CoV-2 infection within 6 months after vaccination. Decline of antibody levels 6 months after vaccination was observed in all groups. Convalescent group A lost more antibodies in the mentioned period of time than group B or C. Group C presented still high results, higher than in group B ($p = 0.007$), but lower than in group A ($p = 0.048$).

Conclusions. Temporary decrease of antibody levels in convalescents after the second dose of SARS-CoV-2 vaccination did not imply further consequences in the form of important antibody level differences 6 months after vaccination. Patients' overweight could have an impact on antibody production but only after natural infection. It did not affect obtained results after vaccinations.

Keywords

SARS-CoV-2 vaccines • COVID-19 • antibody waning • antibody decay • humoral immune response

1. Introduction

The emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in December 2019 has led to the pandemic that engaged many researchers and pharmaceutical companies to establish protective measures as well as to prepare and produce effective vaccines. After a bit more than one year the first vaccine was available, and many people all over the world, especially in well-developed countries, have started vaccination. It has allowed control of the spread of SARS-CoV-2, and helped to save millions of people while reducing the burden of this disease [1].

Vaccine efficacy (VE) was estimated originally for more than 95% of BNT162b2 (Pfizer–BioNTech) in terms of symptomatic Covid-19. However, this has dropped after the Delta variant started to circulate but was still protective (74.2%). There were declines of effectiveness in those admitted to the hospital and older than 65 years of age.

Independently of which kind of vaccine was used, breakthrough infections in vaccinated patients have been observed in those with lower antibody titers [2].

SARS-CoV-2 infection and/or vaccination results in humoral response and the development of neutralizing antibodies

specific for SARS-CoV-2, especially against receptor binding domain (RBD) of the spike (S) protein [3,4].

Neutralizing antibody titers achieved after infection and/or vaccination seem to be an important marker of protection. It is noteworthy to say that WHO has established an international standard for evaluation of the antibody response to COVID-19 vaccines. Use of these standards is intended to contribute to better understanding of the immune response, and particularly of the correlates of protection [5,6].

There are many reports describing the data obtained by the use of the anti-SARS-CoV-2 RBD IgG and SARS-CoV-2 neutralization antibody assays, but in real life commercially available quantitative IgG assays which can reflect neutralization to some extent are more practical, easier to perform, and cheaper [7].

New variants that are emerging are able to evade immunity connected with the original vaccines [8]. It turned out that antibodies produced in response to previous vaccination against SARS-CoV-2 do not provide enough protection against the Omicron variant and its subvariants. Moreover, despite the fact that the new bivalent vaccines used as boosters in previously

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vaccinated individuals have been prepared, they have diminished efficacy against much more transmissible Omicron subvariants – BA.1, BA.4, BA.5, XBB – which have been spreading quickly all over the world [9, 10, 11]

These vaccines are encoding spike proteins both from previous SARS-CoV-2 and Omicron subvariants [12]. One of the explanations of lower efficacy of bivalent vaccines can be the phenomenon called immune imprinting: those initially exposed to the virus present decreased immune response if they meet similar, but not the same virus [13]. Memory B cells which are produced after the first exposure to the virus are able to produce antibodies in a short time after the next contact with the same strain. If it is not the same, mostly antibodies against the previous strain are still generated, but in a significantly lesser amount. Antibodies that exhibit cross-reactivity are produced by the previously generated memory B-cells. Also, IgM are produced to some extent [14]. Some experts suggest that it can make bivalent boosters less effective [15,16]. Also, previous infections with older common seasonal coronaviruses can influence and shape antibody response to SARS-CoV-2 infection and /or vaccination against Covid-19 [17].

It cannot be excluded that immune imprinting can be responsible for the decline of antibody titers after the second dose of vaccine in the convalescents who underwent SARS-CoV-2 infection before vaccination.

We conducted a prospective study evaluating evolution of antibody IgG titers and vaccine efficacy from 0–6 months after 2 doses of BNT162b2 (Pfizer–BioNTech) in 3 groups of adults: naïve, and 2 convalescent groups: one with surprisingly lower antibody concentration after the second shot compared with antibody titers after the first shot, and the second with higher antibody concentration after the second shot of vaccine.

The main aim of the study was to establish the efficacy of full anti-SARS-CoV-2 vaccination and to evaluate antibody levels, especially of the convalescents with antibody decline after the second dose.

2. Materials and methods

2.1. Study Group

This study is a follow-up for the participants of a single-center study performed at the Medical University of Wrocław, Poland, between February 20, 2021 and August 25, 2021 (dates of the follow-up November 17–December 15, 2021). At the very beginning of the project the patients were recruited by an announcement in local media (such as newspapers, television, and the local hospital's website). They filled in a contact form and were called or messaged by members of our team to confirm their willingness to be vaccinated against SARS-CoV-2, to take part in our study, and to initially exclude any contraindications. Later, in

person, the volunteers signed a questionnaire, where they had to provide information about exclusive diseases such as diabetes; any cancer within the last 5 years; chronic kidney, liver, or lung diseases; AIDS or immunosuppression for any other reason. The patients registered for a specific date. Each patient was informed about the aim of the study. Patients were informed that they could withdraw their consent at any stage of the study, and they signed informed consent. It was also necessary for the volunteers to sign in for the vaccination against SARS-CoV-2 before joining the study. In the first part of the study, we assessed anti-SARS-CoV-2 antibody levels before vaccination. Due to frequent changes in registration rules and vaccination dates and patients' individual health contraindications at the moment, the interval between collecting blood samples for testing and the first dose of vaccine varied from 1 day to 6 weeks, usually approximately 1 week.

Later, we invited the patients for antibody level follow-ups after the first dose, precisely up to 7 days before the second vaccination and again 4–5 weeks after the second/last dose. 87 patients were invited for another follow-up approximately 6 months after second dose. In this paper we focus on the results obtained from the last follow-up. These patients were vaccinated only with the Pfizer (Pfizer–BioNTech, BNT162b vaccine).

Inclusion criteria: Patients over 18 years of age who signed informed written consent to participate in the study and were willing to be vaccinated were included. The patients disclosed whether they had had SARS-CoV-2 infection and if it was confirmed with PCR or a serological test. Exclusion criteria: Those who suffered from diabetes; any cancer within the last 5 years; chronic kidney, liver or lung diseases; AIDS or immunosuppression for any other reason were excluded.

Before a blood sample was taken, patients were asked to fill in a questionnaire and to answer questions about their age, weight, height, whether they were ill recently, vaccinated against flu, if they were smoking.

According to the previously established criteria, 87 patients, citizens of Lower Silesia region, Poland, mostly from Wrocław, of both sexes were enrolled to this part of the study. Based on final serological results obtained in earlier stages of the study three groups of patients were established:

- Group A: COVID-19 convalescent with antibody presence in the first stage of this study; with increase of antibody level after second dose (27 people)
- Group B: patients without evidence of previous SARS-CoV-2 infection and with 0 anti-SARS-CoV-2 antibodies before vaccination (naïve patients – 30 people);
- Group C: COVID-19 convalescent with antibody presence in the first stage of this study, convalescents who had lower antibody level after second dose compared to the results after first dose (30 people)

During the study, participants were tested 4 times so far:
D0 (test before vaccination)

D1 (test after first dose – up to 7 days before the second dose)

D2 (test after second dose – 4–5 weeks after the second dose)

D3 (test 6 months after second dose)

All individuals participated in the previous parts of the study, in which we assessed initial antibody titers (before vaccination), also during and shortly after the vaccination. The manuscripts are available online.

2.2. Methods

All 87 participants were tested for the presence of anti-SARS-CoV-2 IgG antibodies. Plasma samples were collected using heparin, centrifuged and stored in aliquots at -70°C for later use. The Anti-SARS-CoV-2 QuantiVac ELISA (IgG) (EUROIMMUN Medizinische Labordiagnostika AG, Luebeck, Germany) was used for quantitative detection of anti-SARS-CoV-2 antibodies by means of 6-point calibration curve.

In the quantitative enzyme-linked immunoabsorbent assay the S1 domain of the spike protein of SARS-CoV-2 including the receptor binding domain (RBD) was used as an antigen. ELISA assay was performed, and the results were evaluated as recommended by the manufacturer. Samples with absorbance higher than the absorbance of the highest standard (386 IU/mL) were diluted and retested. The final results were calculated by multiplying by a dilution factor. The assay was standardized against "First WHO International Standard for anti-SARS-CoV-2 immunoglobulin" (NIBSC 20/136), so the quantitative results are given in standardized units: IU/mL (IU, international units) which are identical to BAU/mL (BAU, binding antibody units).

2.3. Statistical analysis

For each parameter mean, median (M), standard deviation (SD), range (min, max), lower and upper quartile (25Q, 75Q) were calculated. The normality of distribution was tested with the Shapiro-Wilk test. Statistical significance between means for different groups was calculated using the non-parametrical Kruskal-Wallis test (for more than two groups) or Mann-Whitney U test (for two groups). The homogeneity of variance was determined by the Levene's test. The post-hoc comparison was performed for the mean ranks of all pairs of groups.

Statistical significance between frequencies was calculated by the chi-square test with corresponding degree of freedom df ($df = (m-1) * (n-1)$, where m – number of rows, n – number of columns).

The relation between two parameters was assessed using correlation analysis and Spearman correlation coefficients were calculated.

A p value of less than 0.05 was required to reject the null hypothesis. Statistical analysis was performed using EPIINFO Ver. 7.2.4.0 and Statistica Ver. 13.3. software packages.

3. Results

87 participants of the ongoing study on humoral response to SARS-CoV-2 vaccines and their effectiveness were invited for a follow-up visit 6 months after the second dose of the vaccine. Based on previous results, we were able to divide them into 3 groups: (A) convalescents with increase of antibody level after second dose (27 people); (B) naïve patients (30 people); (C) convalescents who had lower antibody level after second dose compared to the results after first dose (30 people). None of the patients reported COVID-19 infection during or after the vaccinations.

While describing individual groups, we were able to exclude some factors from the analysis as we found them to have no statistical importance ($p > 0.05$): patients' sex ($p = 0.258$ ($\chi^2_2 = 2.71$); a diagnosis and new treatment of an acute or chronic disease introduced between second dose and the time of the examination $p = 0.685$ ($\chi^2_2 = 0.76$); smoking $p = 0.300$ ($\chi^2_4 = 4.88$); vaccination against flu during that season $p = 0.243$ ($\chi^2_2 = 2.83$).

Age may be a potentially significant factor regarding groups A and B ($p = 0.030$). Patients of naïve group were slightly younger (median 42.5 vs. 46.0 years old).

While searching for factors which may influence humoral response of the individual groups, various options were taken into consideration. After careful analysis we were able to discover a link between the age and antibody levels before ($p = 0.009$), during ($p = 0.043$), and shortly after vaccination ($p = 0.020$) (D0, D1, D2) but not 6 months later ($p = 0.105$). Although age seems to shape the stimulation strength of the immune system (measured in increasing antibody levels), it did not correlate with the number of remaining memory cells. The most pronounced difference was shown between groups A vs. B ($p = 0.009$), whereas the rest of the comparisons did not bring important information (A vs. C $p = 0.089$; B vs. C $p = 0.532$; Kruskal-Wallis test $p = 0.037$).

Also, the baseline results are important in this matter (see Table 1 and Figure 1).

Table 1. Correlation between baseline (D0) antibody levels and further sampling (D1 – after 1st dose – up to 7 days before the second dose, D2 – 1 month after vaccination, D3 – 6 months after vaccination)

Antibody levels	Spearman rank correlation		
	N	R Spearman	p
D0 & D1	87	0.73	0.000
D0 & D2	87	0.34	0.001
D0 & D3	87	0.45	0.000

Although all groups presented mildly increased BMI or in the high normal range (for group A, median 25.3, IQR [22.5–27.1], minimal-maximal 20.6–38.7; for group B, median 24.4, IQR [21.6–29.4], minimal-maximal 19.5–34.1; for group C, median

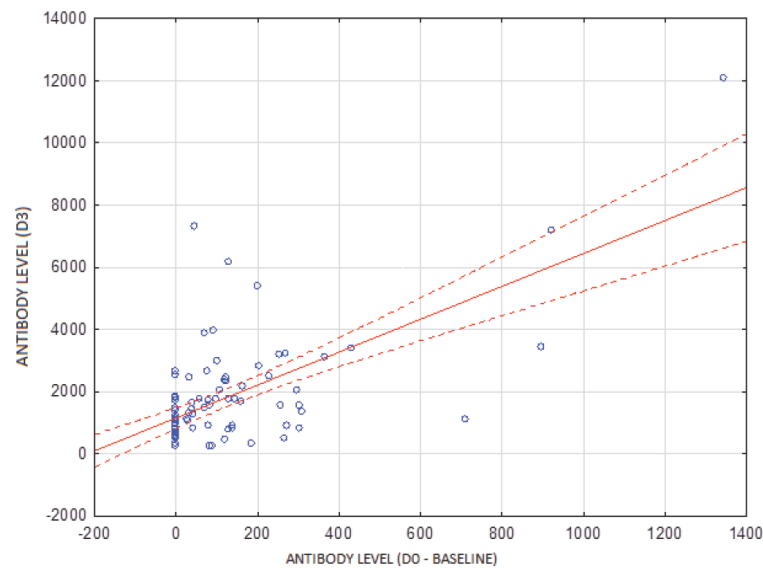


Figure 1. Correlation between baseline (D0) antibody levels and 6 months after vaccination (D3). Blue rings present individual results; red line – 95% confidence interval (CI)

26.0, IQR [23.1-27.9], minimal-maximal 22.5-48.2; in total 25.4, IQR [22.8-27.7]) we were able to identify it as a statistically important factor influencing the antibody levels only before vaccinations (baseline levels; $p = 0.001$). There seems to be a positive correlation between BMI and antibody levels. It may be connected to severity of COVID-19 in the past, although our data from this paper do not allow us to draw further conclusions – only 4 out of 57 convalescents were asymptomatic, leaving 53 patients with mostly mild symptoms of infections who did not require hospitalization.

We did not find a potential correlation between increased BMI and lower antibody levels during or after vaccination at later stages. For detailed information see Figure 2.

In the table below we gathered information regarding all four sample takings and antibody levels for our A, B, and C groups. Before vaccination (D0) the differences between groups of convalescents and naïve patients were clearly visible, whereas we did not observe them between groups A and C (A vs. B $p = 0.0$; B vs. C $p = 0.0$; A vs. C $p = 0.353$). After the first dose (D1) the numbers remained similar (A vs. B $p = 0.0$; B vs. C $p = 0.0$; A vs. C $p = 0.733$). One month after the second dose (D2), when we started to see changes in some of the convalescents resulting in lower antibody levels than before second dose, the statistical analysis showed an important difference between groups A and C and A and B (A vs. B $p = 0.0$; B vs. C $p = 0.633$; A vs. C $p = 0.001$).

The last time the patients were examined, 6 months after the second dose, the differences remained, although they tend to become more like at the beginning on D0 and D1 (A vs. B $p = 0.0$; B vs. C $p = 0.007$; A vs. C $p = 0.048$). We also used the Kruskal-Wallis test to compare all three groups with each other; the result was $p = 0.0$ for all sample takings (D0 H = 53.8; D1 H = 55.8; D2 H = 20.6; D3 H = 22.8). For detailed information see Table 2 and Figure 3.

In this paper we would like to focus mostly on the results obtained during the last testing (D3) and on group C (convalescents with lower antibody levels after the second dose). As described in the previous manuscript, we have seen the decrease of antibody levels after different vaccines but in order to obtain more credible data, for the follow-up we invited only people vaccinated with Pfizer-BioNTech Covid-19 vaccine.

Group C had lower antibody levels after the second dose. Although group C obtained higher results in comparison with other convalescents before and after the first dose, and also in comparison with naïve patients after the second dose, these results were found not to be significantly important ($p = 0.353$; $p = 0.733$; $p = 0.633$ respectively). 6 months later group C presented still high results, higher than in group B ($p = 0.007$), but lower than in group A ($p = 0.048$).

Additionally, we examined the difference (delta) between D2 and D3 (see Table 3 and Figure 4).

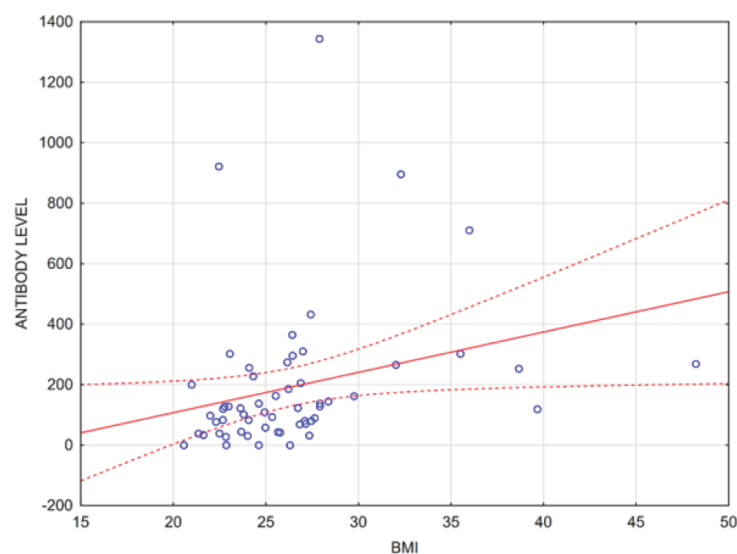


Figure 2. Initial antibody level for groups A (COVID-19 convalescents with increase of antibody level after 2nd dose) and C (COVID-19 convalescent, who had lower antibody level after 2nd dose compared to the results after 1st dose) and BMI level. Blue rings present individual results; red line – 95% confidence interval (CI)

Table 2. Minimal (min), maximal (max), median (Me), IQR (Interquartile range), SD (standard deviation) and mean results [IU/mL] on following stages of the study. A (COVID-19 convalescents with increase of antibody level after 2nd dose) – 27 patients, B (naïve patients) – 30, C (COVID-19 convalescent, who had lower antibody level after 2nd dose compared to the results after 1st dose), – 30; 87 participants in total. P-value was found using Kruskal-Wallis test

	group	Mean [IU/mL]	SD [IU/mL]	Min-max [IU/mL]	Me [IQR] [IU/mL]	P
D0	A	162.4	182.8	0.0-896.0	92.8 [44.2-227.7]	0.000
	C	222.0	289.5	0.0-1344.0	128.1 [83.2-268.8]	
	B	0.000	0.000	0.0-0.0	0.000 [0-0]	
	total	127.0	218.0	0.0-1344.0	57.6 [0.0-144.5]	
D1	A	5959.5	2415.7	1830.4-11392.0	5587.2 [4480.0-7680.0]	0.000
	C	7638.5	5390.0	1968.0-21800.0	5472.0 [4000.0-8256.0]	
	B	856.0	875.1	185.6-3088.0	536.0 [320.0-889.6]	
	total	4778.6	4525.2	185.6-21800.0	4000.0 [777.9-6075.0]	
D2	A	7664.4	2616.5	3360.0-14360.0	7520.0 [4992.0-9280.0]	0.000
	C	5547.2	3845.9	1129.0-19840.0	4608.0 [3120.0-7456.0]	
	B	4451.9	1569.1	1664.0-7680.0	4032.0 [3360.0-5440.0]	
	total	5826.6	3103.9	1129.0-19840.0	4832.0 [3456.0-7536.0]	
D3	A	2417.1	1480.2	344.0-7316.0	2336.0 [1453.0-3096.0]	0.000
	C	2146.4	2453.0	236.0-12099.0	1462.5 [819.0-2177.0]	
	B	965.4	485.8	340.0-2650.0	819.0 [609.0-1216.0]	
	total	1823.2	1781.8	236.0-12099.0	1301.0 [806.0-2336.0]	

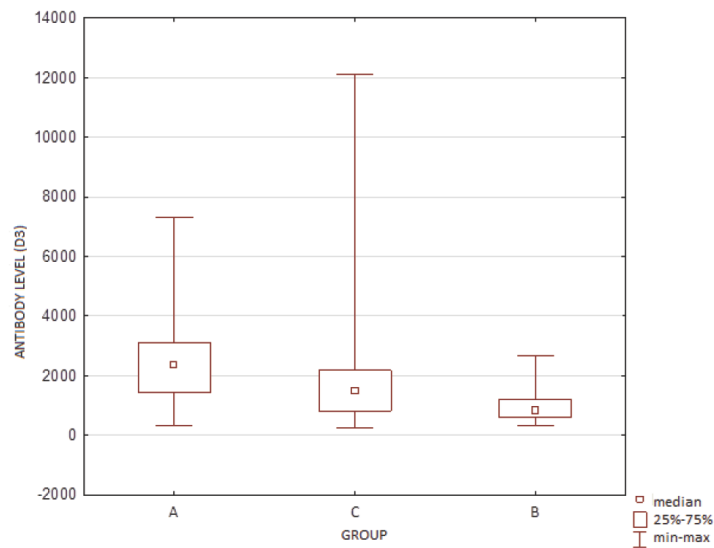


Figure 3. Antibody levels 6 months after vaccination (D3) for group A (COVID-19 convalescents with increase of antibody level after 2nd dose), B (naïve patients) and C (COVID-19 convalescent, who had lower antibody level after 2nd dose compared to the results after 1st dose). Whiskers drawn from minimum (min) to maximum (max).

Table 3. Delta (difference) between antibody levels 1 month after vaccination (D2) and 6 months after vaccination (D3); minimal (min), maximal (max), median (Me), IQR, SD (standard deviation), N – number of subjects, total – summary number of participants. P-value was found using Kruskal-Wallis test

group	Mean [IU/mL]	N	SD [IU/mL]	Min-max [IU/mL]	Me [IQR] [IU/mL]	p
A	5247.2	27	2566.4	1383.0-13459.0	5388.0 [3354.0-7134.0]	0.003
C	3400.8	30	1811.0	756.0-7741.0	3158.5 [2024.0-5012.0]	
B	3486.5	30	1461.3	614.0-6331.0	3105.0 [2654.0-4538.0]	
total	4003.4	87	2126.7	614.0-13459.0	3505.0 [2496.0-5535.0]	

Again, we may observe that the important differences lay between groups A vs. B and A vs. C (U Mann-Whitney test A vs. B $p = 0.003$, $U = 221.0$; B vs. C $p = 0.633$, $U = 417.0$; A vs. C $p = 0.003$, $U = 223.0$; Kruskal-Wallis test $H = 11.4$, $p = 0.003$). Convalescents group A lost more antibodies in the mentioned period of time than group B or C.

We also asked if the results obtained after the second dose led to any decisions regarding a third dose. Most of the participants were willing to sign in for the third dose in the recommended time (6-12 months after second dose) – A-15; B-21, C-17; in total 53/87 (60.91%), but interestingly many responders decided to make the decision dependent on the recent results: A-11, B-7, C-12, in total 30/87 (34.48%). Only 2 patients planned to wait more than 12 months (A-1, C-1) and other 2 declared to abstain from the third dose (B-2).

4. Discussion

In our study initial antibody levels dwindle, which is a fact reported by multiple researchers (citations below). It was observed in all 3 study groups. However, the most important drop in the antibody levels 6 months after vaccination was seen in convalescents group A [difference D2-D3 mean 5247.2 IU/mL, min-max 1383.0-13459.0]. The difference result for group B (naïve) and C (convalescents with antibody drop after second dose) were similar [delta D2-D3 mean 3486.5 vs. 3400.8 IU/mL; min-max 614.0-6331.0 vs. 756.0-7741.0 respectively]. The final mean antibody levels 6 months after vaccination were: A-2417.1; B-965.4; C-2146.4 IU/mL, meaning the highest decrease of antibodies was observed in the naïve patients group (4.5-fold decrease). In the modeling of Pérez-Alós L. et al. the decline was also more pronounced in those previously non-infected;

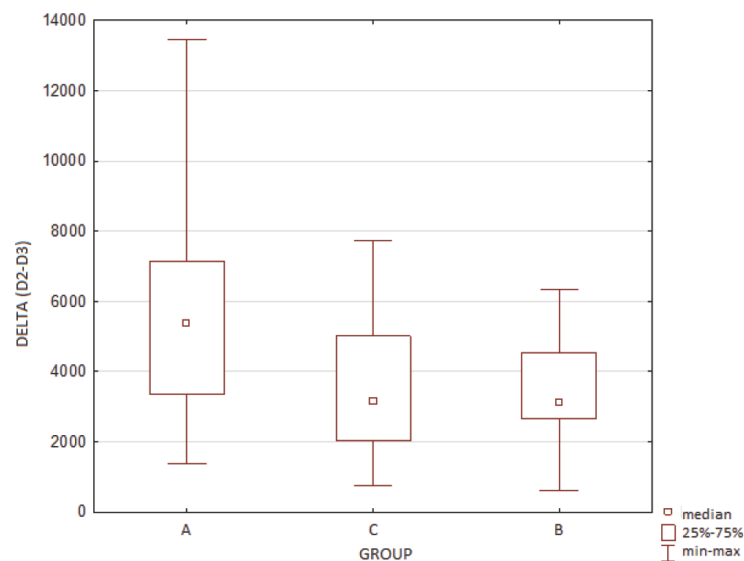


Figure 4. Delta (difference) between antibody levels 1 month after vaccination (D2) and 6 months after vaccination (D3)

moreover, similarly they report that natural infections prior to completion of vaccination induced a more robust immune response, but humoral responses following COVID-19 vaccination decreased in all age groups after approximately 6 months [18]. Also in studies focused on RBD-binding IgG antibody levels the constant decrease among vaccinated people is reported and convalescents tend to have higher antibody titers than naïve patients at the beginning of the study, as well as in the final check-up [19].

M. Skorupa et al. describes a partially similar study [20]. They used the same assay as we did. The changes in the level of antibodies were most dynamic during the first 3 months. In the first month after taking the second dose of BNT162b, the level of antibodies increased intensively, followed by a systematic decrease in the concentration of anti-SARS-CoV-2 IgG. Skorupa et al. observed that 6 months after vaccination a group of patients without prior infection had still high antibody titers compared with the rest of vaccinated individuals. The explanation is that they probably had an asymptomatic infection after full vaccination. It was observed that vaccination can diminish the symptoms or lead to asymptomatic infection with SARS-CoV-2.

Luczkowiak J et al. reported 6.3-fold decline in the neutralizing activity in the cohort of vaccinated individuals 8 months earlier with BNT162b2 vaccine, both naïve and convalescents, however,

the mean NT50 titer in the convalescents was higher (839 vs 118 GMT) [21]. There was a significant proportion (19%) of naïve individuals without detectable neutralization activity after 8 months.

Levin et al., in the prospective large-scale, real-world, longitudinal study among naïve health care workers under observation till 6 months after receipt of the second dose of the BNT162b2 vaccine, reported a decrease of IgG levels against SARS-CoV-2 at a consistent rate but with a significant drop of neutralizing antibody levels during the first 3 months after vaccination [22]. Then the decline was slower. Moreover, neutralizing antibody levels in the sixth month differed between men and women, were lower in men, and also in persons 65 years of age and older.

Also in the large-scale study, through 6 months of follow-up among naïve participants of multinational population who got 2 doses of BNT162b2, it was shown that the vaccine was highly effective (86 to 100%) although the efficacy was gradually declining across the continents, countries, races, etc. [23].

We did not observe any Covid-19 within 6 months after vaccination in either group, naïve or convalescent. It cannot be excluded that some individuals were infected asymptotically, since these infections usually are not characterized by the severe course of the disease, as reported by Singanayagam, Hakki, Dunning, et al. [24]

At first glance, lower antibody results after the second dose in some convalescents may look disappointing or even alarming – the aim of administering the second doses was contrary to the actual result. The further follow-up showed some calming data which we would like to discuss in the light of the latest news on the topic. In our study the antibody levels after the second dose of vaccine were significantly lower in a group of 30/87 individuals (34.48% of convalescents). The immune imprinting concept cannot be excluded as the explanation of our results in the real-life study.

The phenomenon was described for the first time in 1947 in terms of antibody response after subsequent flu infections [25].

This immune imprinting was also noticed before Omicron occurrence in those who were vaccinated against Covid-19 and previous SARS-CoV-2 infection. The magnitude of immune response depended on earlier infection or vaccination. Reynolds et al. reported that after infection and two vaccine shots S1 antibody, memory B cells, heterologous neutralization of B.1.351, P.1, and B.1.617.2 plateaued, but B.1.1.7 neutralization and spike T cell responses increased. [12].

Marzi et al. observed in previously infected individuals that the serum antibody levels reached the highest results after the first shot, and no further increase was seen after the second or the third dose [26]. Moreover, when looking at the avidity of SARS-CoV-2 S- and RBD-specific serum antibodies they found rapid increase of the avidity in naive donors while in infected individuals it was high before vaccination and did not increase over time.

The question is why lower antibody levels affected only a part of the convalescent individuals after the second dose of vaccine. Seasonal coronaviruses are a common reason of many infections among the population observed every year. Did the previous infections with seasonal coronaviruses influence antibody response to SARS-CoV-2 vaccination against Covid-19? Unfortunately, we did not check antibodies against OC43, HKU1, and 229E seasonal coronaviruses. Aydiillo T et al. tested humoral response due to SARS-CoV-2 in patients suffering from Covid-19 as well as pre-existing immunity to OC43, HKU1, and 229E seasonal coronaviruses. There was back-boosting effect to conserved regions of OC43 and HKU1 betacoronaviruses spike protein. The authors suggested that this could negatively influence production of IgG and IgM against the SARS-CoV-2 spike and nucleocapsid protein [17].

Our small observation can confirm the idea of immune imprinting. Large studies are needed to analyze the real significance of this phenomenon in the development of future vaccines, not only those directed against Covid-19.

One could expect better protection than this achieved after vaccination with bivalent boosters against Covid-19, which revealed a modest increase of antibody levels (BA.4 and BA.5). The data of immune imprinting are important in terms of future

vaccine creation potent against emerging variants of SARS-CoV-2 [27].

In our cohort we observed significant differences in older age in terms of antibody production after COVID-19 (D0), after the first (D1) and second dose (one month after; D2), but later on, after 6 months the age seemed not to remain as an important factor. There are some known reports about weaker immune response after the vaccination and faster antibody waning in older people [28,29].

Also H.S. Yang et al. came to interesting conclusions while comparing a large group of pediatric patients to young adults (1194 pediatric patients [mean (SD) age, 11.0 (5.3) years] and 30,232 adult patients) [30]. Children exhibited higher median (IQR) IgG levels, TAB (total antibody levels), and SNAb (surrogate neutralizing antibody) activity compared with adolescents (e.g., IgG levels: 473 [233-656] RFU vs 191 [82-349] RFU; $P < .001$) and young adults (e.g., IgG levels: 473 [233-656] RFU vs 85 [38-150] RFU; $P < .001$). In our study we recruited only adults aged 18 or older.

Opposite to our findings, Szczepanek et al. concluded that anti-SARS-CoV-2 concentrations were correlated only with earlier infection; they did not identify any link between antibody levels, gender, and age [31].

As many authors mentioned above and more, we decided to measure anti-spike IgG levels, as it is a method easily accessible, relatively cheap, and could be widely used not only in study programs but also in real-life everyday clinical practice.

Assuming that patients' BMI was relatively stable during the months between each blood examination, we can conclude that being overweight (BMI 25.0-29.9) had some important influence on antibody levels produced or remaining months after SARS-CoV-2 infection, causing higher results in comparison with other convalescents.

Obesity is a problem that scientists are concerned with in many fields. Also, regarding humoral response after SARS-CoV-2 infections or vaccination, patients' weight was a factor of interest. Arwa A. Faizo et al. presented the prevalence of COVID-19 neutralizing antibodies among control (normal BMI) and study (obese with BMI ≥ 30 kg/m²) groups after two doses of the vaccine, showing higher antibody value in control versus the study group [32]. Compared with normal weight group, significantly more people with severe obesity six months after their second vaccine dose, in the study of A. van der Klaauw et al., had, among others, unquantifiable titers of neutralizing antibody against authentic SARS-CoV-2 virus (NABs) [33]. Although obesity is a well-known factor which may compromise the immune system to some limited level, there are a few reports concerning overweight, such as the one of Qian Zhu et al., who examined individuals with obesity/overweight (BMI >24) and concluded that they had a reduced seropositivity rate of NABs compared to those with normal BMI; anti-RBD-IgG and

NAbs titers in the high BMI group were significantly lower than those in the normal BMI group [34].

Contrary to our findings, in 17 out of 23 studies of a systematic review of epidemiological studies analyzing the effect of smoking on humoral response, the researchers concluded that current smokers showed much lower antibody titers or more rapid lowering of the vaccine-induced IgG compared with nonsmokers [35]. Emerging evidence has described lower antibody levels in response to COVID-19 mRNA vaccine in smokers, irrespective of duration of smoking or number of cigarettes per day [36]. The differences in our study may be partially explained by the fact that the numbers in our study refer mostly to smoking in the past (only 2 active smokers, 18 who quit; 67 who never smoked).

Surprisingly there was evidence of positive influence of influenza vaccine on humoral response to SARS-CoV-2 vaccine. Data published by Poniedziałek et al. showed that the influenza-vaccinated group of patients had significantly higher frequency and titers of anti-N antibodies (75% vs. 66%; mean 559 vs. 520 U/mL) and anti-RBD antibodies (85% vs. 76%; mean 580 vs. 540 U/mL) [37]. Our results do not match; however, our group of patients vaccinated against influenza was not sizeable (11/87 patients) and refers to flu vaccination after 2 doses of anti-SARS-CoV-2 shots (while Poniedziałek et al. examined patients vaccinated against flu in the 2019/2020 season).

5. Conclusions

Independently of previous differences due to antibody levels both in naïve and convalescent individuals, none of the patients suffered from symptomatic SARS-CoV-2 infection within 6 months after vaccination.

Temporary decrease of antibody levels in convalescents after the second dose of SARS-CoV-2 vaccination did not imply further consequences in the form of important antibody level differences 6 months after vaccination.

Further investigation concerning immune response differences in terms of immune imprinting should be performed in large groups of patients vaccinated and infected with subsequent viral variants.

Patients' overweight could have an impact on antibody productions but only after natural infection; it did not affect obtained results after vaccinations.

6. Limitations

This stage of the study takes ended shortly before the Omicron variant spread in Poland and rest of Europe.

During the study, the patients were not tested for SARS-CoV-2 infection between the blood sampling, therefore one may not exclude that asymptomatic infections occurred.

Small study group can influence the results.

Abbreviations

BMI – body mass index; SARS-CoV-2 – severe acute respiratory syndrome coronavirus 2; WHO – World Health Organization.

Ethic approval

The study received approval of the Bioethic Committee at Wrocław Medical University (No 51/2021). Informed consent was obtained from all subjects involved in the study.

Authors' contribution

Conceptualization, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; methodology, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; software, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; validation, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; formal analysis, M.S., M.Z., B.K. investigation, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; resources, M.Z., B.K., B.J-P., A.P., A.K.; data curation, M.S., B.K., M.Z.; writing—original draft preparation, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; writing—review and editing, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; visualization, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; supervision, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; project administration, M.S., M.Z., B.K., N. Ś-L., B.J-P., A.P., A.K.; funding acquisition, M.Z., B.K., B.J-P., A.P., A.K. All authors have read and agreed to the published version of the manuscript

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Conflict of interest

The authors have no potential conflicts of interest to declare.

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- [37] Poniedziałek B, Hallmann E, Sikora D, Szymański K, Kondratiuk K, Żurawski J, Rzymiski P, Brydak L. Relationship between Humoral Response in COVID-19 and Seasonal Influenza Vaccination. *Vaccines*. 2022;10:1621.

XII. Załączniki

a) Oświadczenia współautorów publikacji

Lek. Monika Stępień
Katedra i Klinika Chorób Zakaźnych, Chorób Wątroby
i Nabytych Niedoborów Odpornościowych
Uniwersytet Medyczny we Wrocławiu

Wrocław, 29.01.2024 r.

OŚWIADCZENIE

Oświadczam, że w 3 publikacjach:

- 1) Monika Stępień, Natalia Świątoniowska-Lonc, Brygida Knysz, Beata Jankowska-Polańska, Amadeusz Kuźniarski, Agnieszka Piwowar, Małgorzata Zalewska; 2023; '*An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland*'; Advances in Clinical and Experimental Medicine
- 2) Monika Stępień, Małgorzata Zalewska, Brygida Knysz, Natalia Świątoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łacmański, Agnieszka Piwowar, Amadeusz Kuźniarski; 2022; '*How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection*'; Vaccines
- 3) Monika Stępień, Małgorzata Zalewska, Amadeusz Kuźniarski, Beata Jankowska-Polańska, Agnieszka Piwowar, Natalia Świątoniowska-Lonc, Brygida Knysz; 2023; '*How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2*'; Advances in Hygiene and Experimental Medicine

mój udział polegał na głównej roli w stworzeniu projektu badania, kontakcie bezpośrednim z pacjentami w trakcie rekrutacji oraz na wszystkich późniejszych etapach, na tworzeniu baz danych, interpretacji danych statystycznych i opracowaniu wyników, stworzeniu pierwotnych i ostatecznych wersji manuskryptów.

potwierdzenie
prof. dr hab. Brygida Knysz

Monika Stępień

Prof. dr hab. n. med. Brygida Knysz
Katedra i Klinika Chorób Zakaźnych, Chorób Wątroby
i Nabytych Niedoborów Odpornościowych
Uniwersytet Medyczny we Wrocławiu

Wrocław, 29.01.2024 r.

OŚWIADCZENIE

Oświadczam, że w 3 publikacjach:

- 1) Monika Stępień, Natalia Świątoniowska-Lonc, Brygida Knysz, Beata Jankowska-Polańska, Amadeusz Kuźniarski, Agnieszka Piwowar, Małgorzata Zalewska; 2023; '*An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland*'; Advances in Clinical and Experimental Medicine
- 2) Monika Stępień, Małgorzata Zalewska, Brygida Knysz, Natalia Świątoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łączmański, Agnieszka Piwowar, Amadeusz Kuźniarski; 2022; '*How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection*'; Vaccines
- 3) Monika Stępień, Małgorzata Zalewska, Amadeusz Kuźniarski, Beata Jankowska-Polańska, Agnieszka Piwowar, Natalia Świątoniowska-Lonc, Brygida Knysz; 2023; '*How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2*'; Advances in Hygiene and Experimental Medicine

mój wkład polegał na współuczestnictwie w opracowaniu koncepcji i metodologii badania, w stworzeniu ostatecznej wersji manuskryptów, ich recenzji i edycji oraz krytycznej ocenie prac.

Potwierdzenie
Katedra i Klinika Chorób Zakaźnych, Chorób Wątroby
i Nabytych Niedoborów Odpornościowych
Uniwersytet Medyczny we Wrocławiu
prof. dr hab. Brygida Knysz

Uniwersytet Medyczny we Wrocławiu
Katedra i Klinika Chorób Zakaźnych, Chorób Wątroby
i Nabytych Niedoborów Odpornościowych
prof. dr hab. Brygida Knysz

Dr hab. Beata Jankowska-Polańska, prof. PWR
Dyrektor Centrum Wsparcia Badań Klinicznych
4 Wojskowy Szpital Kliniczny z Polikliniką SPZOZ we Wrocławiu

Wrocław, 22.01.2024 r.

OŚWIADCZENIE

Oświadczam, że w 3 publikacjach:

- 1) Monika Stępień, Natalia Świątoniowska-Lonc, Brygida Knysz, Beata Jankowska-Polańska, Amadeusz Kuźniarski, Agnieszka Piwowar, Małgorzata Zalewska; 2023; '*An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland*'; Advances in Clinical and Experimental Medicine
- 2) Monika Stępień, Małgorzata Zalewska, Brygida Knysz, Natalia Świątoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łaczmanski, Agnieszka Piwowar, Amadeusz Kuźniarski; 2022; '*How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection*'; Vaccines
- 3) Monika Stępień, Małgorzata Zalewska, Amadeusz Kuźniarski, Beata Jankowska-Polańska, Agnieszka Piwowar, Natalia Świątoniowska-Lonc, Brygida Knysz; 2023; '*How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2*'; Advances in Hygiene and Experimental Medicine

mój udział polegał na współuczestnictwie w opracowaniu koncepcji badań, w organizowaniu środków finansowych, w analizie danych statystycznych, ponadto uczestniczyłam w weryfikacji spójności i akceptacji ostatecznej wersji każdego manuskryptu.

Podpis

Polina chow

Jankowska-Polańska

B. Knysz

prof. dr hab. n. med. Brygida Knysz
lekarz chorób wewnętrznych
specjalista chorób zakaźnych

3624440

dr n. biol. Małgorzata Zalewska
Katedra i Klinika Chorób Zakaźnych, Chorób Wątroby
i Nabytych Niedoborów Odpornościowych
Uniwersytet Medyczny we Wrocławiu

Wrocław, 07.12.2023 r.

OŚWIADCZENIE

Oświadczam, że w 3 publikacjach:

- 1) Monika Stępień, Natalia Świątoniowska-Lonc, Brygida Knysz, Beata Jankowska-Polańska, Amadeusz Kuźniarski, Agnieszka Piwowar, Małgorzata Zalewska; 2023; '*An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland*', Advances in Clinical and Experimental Medicine
- 2) Monika Stępień, Małgorzata Zalewska, Brygida Knysz, Natalia Świątoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łaczmanski, Agnieszka Piwowar, Amadeusz Kuźniarski; 2022; '*How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection*'; **Vaccines**
- 3) **Monika** Stępień, Małgorzata Zalewska, Amadeusz Kuźniarski, Beata Jankowska-Polańska, Agnieszka Piwowar, Natalia Świątoniowska-Lonc, Brygida Knysz; 2023; '*How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2*'; Advances in Hygiene and Experimental Medicine

mój udział polegał na wykonaniu testów anty-SARS-CoV-2 QuantiVac ELISA IgG z próbek krwi pobranych od pacjentów na 3 etapach badania, uwzględnionych w powyższych publikacjach oraz na opisanu metodologii wykonanych badań serologicznych (jako części paragrafu Material and methods); ponadto uczestniczyłam w weryfikacji spójności i akceptacji ostatecznej wersji każdego manuskryptu.

potwierdza

B. Knysz

prof. dr hab. n. med. Brygida Knysz
lekarz chorób wewnętrznych
specjalista chorób zakaźnych

3624440

Małgorzata Zalewska

Dr hab. Łukasz Łaczmąński, prof. IITD PAN
Laboratorium Genomiki i Bioinformatyki
Instytut Immunologii i Terapii Doświadczalnej
Im. Ludwika Hirszfelda Polskiej Akademii Nauk

Wrocław, 3.01.2024

OŚWIADCZENIE

Oświadczam, że w publikacji:

Monika Stępień, Małgorzata Zalewska, Brygida Knysz, Natalia Świętoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łaczmąński, Agnieszka Piwowar, Amadeusz Kuźniarski; 2022; *'How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection'*; Vaccines

mój udział polegał na analizie danych statystycznych, na przygotowaniu tabeli nr 6 oraz ryciny nr 1, ponadto uczestniczyłem w weryfikacji spójności i akceptacji ostatecznej wersji manuskryptu.

Podpis

dr hab. Łukasz Łaczmąński
Kierownik Laboratorium
Genomiki i Bioinformatyki

potwierdzenie

B. Knysz
prof. dr hab. n. med. Brygida Knysz
lekarz chorób wewnętrznych
specjalista chorób zakaźnych
3624440

Prof. dr hab. Agnieszka Piwowar
Katedra i Zakład Toksykologii,
Uniwersytet Medyczny we Wrocławiu

Wrocław, 08.01.2024

OŚWIADCZENIE

Oświadczam, że w 3 publikacjach:

- 1) Monika Stępień, Natalia Świątoniowska-Lonc, Brygida Knysz, Beata Jankowska-Polańska, Amadeusz Kuźniarski, Agnieszka Piwowar, Małgorzata Zalewska; 2023; *'An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland'*; Advances in Clinical and Experimental Medicine
- 2) Monika Stępień, Małgorzata Zalewska, Brygida Knysz, Natalia Świątoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łaczmarski, Agnieszka Piwowar, Amadeusz Kuźniarski; 2022; *'How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection'*; Vaccines
- 3) Monika Stępień, Małgorzata Zalewska, Amadeusz Kuźniarski, Beata Jankowska-Polańska, Agnieszka Piwowar, Natalia Świątoniowska-Lonc, Brygida Knysz; 2023; *'How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2'*; Advances in Hygiene and Experimental Medicine

mój udział polegał na współorganizowaniu funduszy niezbędnych do przeprowadzenia badania, na analizie danych statystycznych, ponadto uczestniczyłam w weryfikacji spójności i akceptacji ostatecznej wersji każdego manuskryptu.

potwierdzenie

B. Knysz

prof. dr hab. n. med. Brygida Knysz
lekarz chorób wewnętrznych
specjalista chorób zakaźnych

3624440

Podpis

A. Piwowar

Amadeusz Kuźniarski
Katedra i Zakład Protetyki Stomatologicznej
Uniwersytet Medyczny im. Piastów Śląskich
we Wrocławiu

Wrocław, 10.01.2024r.

Oświadczam, że w 3 publikacjach:

- 1) Monika Stępień, Natalia Świętoniowska-Lonc, Brygida Knysz, Beata Jankowska-Polańska, Amadeusz Kuźniarski, Agnieszka Piwowar, Małgorzata Zalewska; 2023; *'An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland'*; Advances in Clinical and Experimental Medicine
- 2) Monika Stępień, Małgorzata Zalewska, Brygida Knysz, Natalia Świętoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łaczmański, Agnieszka Piwowar, Amadeusz Kuźniarski; 2022; *'How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection'*; Vaccines
- 3) Monika Stępień, Małgorzata Zalewska, Amadeusz Kuźniarski, Beata Jankowska-Polańska, Agnieszka Piwowar, Natalia Świętoniowska-Lonc, Brygida Knysz; 2023; *'How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2'*; Advances in Hygiene and Experimental Medicine

mój udział polegał na organizacji lokalu, w którym pacjenci mogli oddać krew po uprzednim zakwalifikowaniu do badania, zajmowałem się transportem materiałów medycznych, ponadto uczestniczyłem w weryfikacji spójności i akceptacji ostatecznej wersji każdego manuskryptu.

potwierdza

B. Knysz

prof. dr hab. o med. Brygida Knysz
lekarz chorób wewnętrznych
specjalista chorób zakaźnych

3624440

Podpis



Natalia Świątoniowska-Lonc
Klinika Kardiologii
4 Wojskowy Szpital Kliniczny
z Polikliniką SP ZOZ we Wrocławiu

Wrocław, 03.01.2024

OŚWIADCZENIE

Oświadczam, że w 3 publikacjach:

- 1) Monika Stępień, Natalia Świątoniowska-Lonc, Brygida Knysz, Beata Jankowska-Polańska, Amadeusz Kuźniarski, Agnieszka Piwowar, Małgorzata Zalewska; 2023; *'An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland'*; Advances in Clinical and Experimental Medicine
- 2) Monika Stępień, Małgorzata Zalewska, Brygida Knysz, Natalia Świątoniowska-Lonc, Beata Jankowska-Polańska, Łukasz Łaczmański, Agnieszka Piwowar, Amadeusz Kuźniarski; 2022; *'How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection'*; Vaccines
- 3) Monika Stępień, Małgorzata Zalewska, Amadeusz Kuźniarski, Beata Jankowska-Polańska, Agnieszka Piwowar, Natalia Świątoniowska-Lonc, Brygida Knysz; 2023; *'How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2'*; Advances in Hygiene and Experimental Medicine

mój udział polegał na pobieraniu próbek krwi pacjentów zakwalifikowanych do każdego etapu badania, na stworzeniu bazy danych na podstawie ankiet pacjentów, na analizie danych statystycznych, ponadto uczestniczyłam w weryfikacji spójności i akceptacji ostatecznej wersji każdego manuskryptu.

Świątoniowska-Lonc Natalia

*podpis
B. Knysz*

prof. dr hab. n. med. Brygida Knysz
lekarz chorób wewnętrznych
specjalista chorób zakaźnych

3624440

b) Nota biograficzna autora

Urodziłam się 25 maja 1992 r. w Świebodzicach. W latach 2014-2020 studiowałam na kierunku lekarskim Uniwersytetu Medycznego we Wrocławiu, w tym czasie odbyłam praktyki wakacyjne w formie stażu na Oddziale Pediatrycznym Chorób Zakaźnych i Tropikalnych w Sardjito General Hospital, Yogyakarta, Indonezja (sierpień 2018 r.) oraz na Oddziale Chorób Zakaźnych i Tropikalnych szpitala Pitié Salpêtrière, Paryż, Francja (sierpień 2019 r.). W 2018 r. otrzymałam certyfikat „ESME in Medical Education” po ukończeniu AMEE ESME Student Online Course. W 2020 r. zakończyłam studia uzyskując tytuł lekarza, a po odbyciu stażu podyplomowego, rozpoczęłam szkolenie specjalizacyjne w ramach rezydentury w II Oddziale Chorób Zakaźnych w WSS im. Gromkowskiego we Wrocławiu. W październiku 2020 r. rozpoczęłam pracę jako asystent w Katedrze i Klinice Chorób Zakaźnych, Chorób Wątroby i Nabytych Niedoborów Odpornościowych Uniwersytetu Medycznego we Wrocławiu, pod kierownictwem prof. dr hab. Brygidy Knysz. W 2021 r. angażowałam się w pracę punktów szczepień jako lekarz kwalifikujący do szczepień przeciwko SARS-CoV-2. Od 2022r. pracuję również jako konsultant ds. anonimowego testowania w kierunku HIV w Punkcie Konsultacyjno-Diagnostycznym Wrocławskiego Centrum Zdrowia SP ZOZ.

Swoją wiedzę pogłębiłam na konferencjach naukowych, szkoleniach oraz kursach dotyczących m.in. ultrasonografii jamy brzusznej. Jestem członkiem Polskiego Towarzystwa Epidemiologów i Lekarzy Chorób Zakaźnych, Polskiego Towarzystwa Naukowego AIDS i Polskiego Towarzystwa Wakcynologii.

c) Wykaz publikacji autora

1. Publikacje w czasopismach naukowych

Lp.	Opis bibliograficzny	IF	Punkty
1	Stępień Monika, Zalewska Małgorzata, Knysz Brygida, Świątoniowska-Lonc Natalia, Jankowska-Polańska Beata, Łaczmański Łukasz, Piwowar Agnieszka, Kuźniarski Amadeusz: How humoral response and side effects depend on the type of vaccine and past SARS-CoV-2 infection, <i>Vaccines</i> , 2022, vol. 10, nr 7, art.1042 [16 s.], DOI:10.3390/vaccines10071042	7,8	140
2	Szymańska Beata, Stępień Monika, Knysz Brygida, Zalewska Małgorzata, Radziszewski Dawid, Cieplucha Hubert, Szetela Bartosz, Szymczak Aleksandra, Piwowar Agnieszka: Evaluation of immune status and selected sirtuins in people living with HIV after fully vaccinating for COVID-19, <i>Acta Poloniae Pharmaceutica</i> , 2023, vol. 80, nr 6, s. 1011-1028, DOI:10.32383/appdr/175542	0,4*	140
3	Stępień Monika, Świątoniowska-Lonc Natalia, Knysz Brygida, Jankowska-Polańska Beata, Kuźniarski Amadeusz, Piwowar Agnieszka, Zalewska Małgorzata: An epidemiological and retrospective study in a cohort qualified for SARS-CoV-2 vaccination in the region of Lower Silesia, Poland [research letter], <i>Advances in Clinical and Experimental Medicine</i> , 2023, vol. 32, nr 3, s. 385-389, DOI:10.17219/acem/161461	2,1*	140
4	Saden Gabrielle, Jastrzębska Aleksandra, Knysz Brygida, Stępień Monika: HIV infection in a woman with Mayer-Rokitansky-Küster-Hauser syndrome - psychological and clinical implications: a case report and literature review, <i>HIV and AIDS Review</i> , 2023, vol. 22, nr 3, s. 269-273, DOI:10.5114/hivar.2023.131630	0,2*	40
5	Stępień Monika, Zalewska Małgorzata, Kuźniarski Amadeusz, Jankowska-Polańska Beata, Piwowar Agnieszka, Świątoniowska-Lonc Natalia, Knysz Brygida: How many is good enough? An analysis of serological follow-up after vaccination against SARS-CoV-2, <i>Postępy Higieny i Medycyny Doświadczalnej</i> , 2023, vol. 77, nr 1, s. 143-153, DOI:10.2478/ahem-2023-0020	0,3*	40
	Podsumowanie	10,8	500

*IF 2022

2. Monografie naukowe - rozdziały

Lp.	Opis bibliograficzny	Punkty
1	Stępień Monika, Kirsch Joanna, Skibińska Dominika, Szymańska Aleksandra, Walkowiak Mateusz, Matkowska-Kocjan Agnieszka: Niepożądane odczyny poszczepienne jako czynnik ryzyka nieprawidłowej realizacji dalszych szczepień, W: Szczepienia ochronne w badaniach naukowych i praktyce lekarskiej, (red.) Jacek Tabarkiewicz, Paulina Frączek, Rzeszów 2019, Wydawnictwo Uniwersytetu Rzeszowskiego, s. 119-127, ISBN 978-83-7996-773-5	20
	Podsumowanie	20