“CAROL DAVILA” UNIVERSITY

OF MEDICINE AND PHARMACY,

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FIELD: MEDICINE

EXPLORING THE IMMUNE RESPONSE IN SARS-CoV-2 INFECTION

SUMMARY OF THE DOCTORAL THESIS

Thesis Advisor

PROF. UNIV. DR. HRISTEA ADRIANA

Doctoral Student:

NEDELCU IULIA-MARIA

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Table of contents

Introduction

The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has triggered a global public health crisis and significantly impacted the economy, society, and global policy. Understanding the immune response to SARS-CoV-2 infection has become essential for developing effective diagnostic, prevention, and treatment solutions.

The novelty and relevance of this research are supported by the continuous evolution of the pandemic, the emergence of new viral strains, and rapid changes in medical practices and public health policies.

The research undertaken in this thesis is part of global efforts to combat the COVID-19 pandemic. The international, national, and regional relevance of this topic is reflected in the active involvement of numerous research teams worldwide, striving to uncover pathogenic mechanisms and develop effective therapies and vaccines. Our research team, along with local collaborators, contributes to the collective effort to understand and combat this devastating disease.

The central hypothesis of this thesis is that the immune response to SARS-CoV-2 infection varies significantly based on genetic and demographic factors, influencing disease severity and treatment efficacy.

The scientific objectives addressed in this thesis include evaluating the performance of different commercially available serological tests, analyzing seroconversion and antibody dynamics in response to SARS-CoV-2 infection, and assessing the neutralizing capacity of antibodies against specific viral variants. Additionally, the study investigates risk factors associated with Long COVID, providing a comprehensive overview of the immune response and disease progression in the context of the COVID-19 pandemic.

This interdisciplinary research combines fields such as immunology, molecular biology, genetics, and clinical practice. Collaboration among experts in these domains has enabled a holistic approach to addressing the complex issues related to the immune response to SARS-CoV-2.

Personal contributions

1. General objectives

This thesis aims to deepen the understanding of the immune response in SARS-CoV-2 infection by evaluating serological tests, analyzing the dynamics and neutralization capacity of antibodies against viral variants, and studying factors influencing disease progression, including Long COVID.

Summary of the three studies conducted during the doctoral research, along with the formulated objectives, is illustrated in Figure 1.

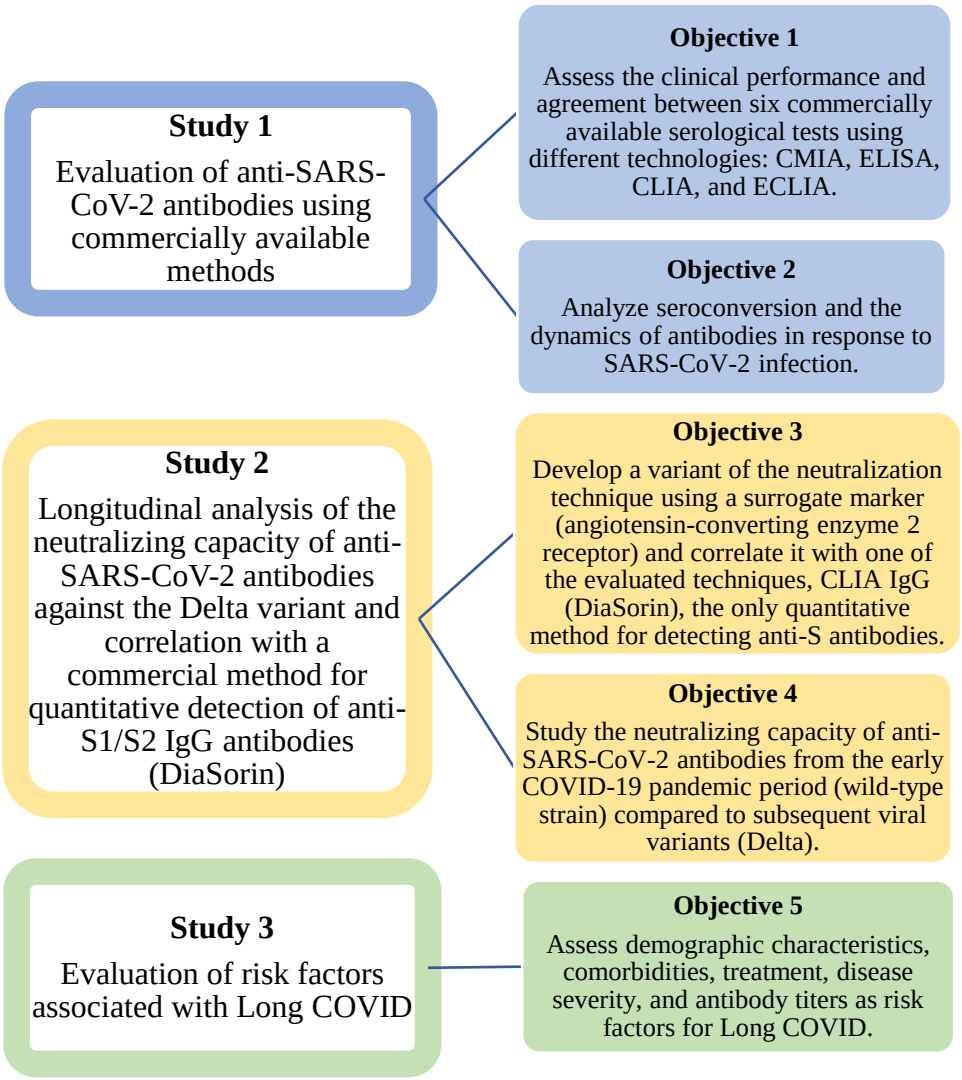

Figure 1. Plan for doctoral research study

2. Materials and methods for studies 1 and 2

The first study was a prospective multicenter study conducted in two hospitals in Bucharest, Romania, and focused on the serological response to SARS-CoV-2 infection. The patient enrollment period spanned May to October 2020, involving 156 patients presenting with varying disease severities. Serial serum samples were collected to analyze the dynamics of antibody production. Six commercially available serological tests were used to detect IgM and IgG antibodies: Elecsys® IgM and IgG anti-SARS-CoV-2 (Roche), LIAISON® SARS-CoV-2 S1/S2 IgG (DiaSorin), SARS-CoV-2 IgG and IgM (Abbott), and ELISA kits KT-1032 EDI™ IgG and KT-1033 EDI™ IgM (Epitop). Statistical analysis assessed the sensitivity, specificity, and concordance among these serological methods.

The second study included patients with symptomatic forms of COVID-19, monitored through serum sample collection at 30, 90, and 180 days from the onset of symptoms, excluding patients who were vaccinated at any point during the study.. The objective was to monitor specific antibody responses and neutralizing antibodies using both chemiluminescence testing and pseudoneutralization assays. Statistical analyses evaluated antibody dynamics and correlations between the testing methods over time.

3. Evaluation of anti-SARS CoV-2 antibodies using commercially available methods

3.1. Objective 1: Clinical performance and concordance between six commercially available serological tests using different technologies: ELISA, CMIA, CLIA, and ECLIA

3.1.1. Introduction

With the evolution of the COVID-19 pandemic, research on the immune response in SARS-CoV-2 infection has advanced significantly (1). However, immunity remains incompletely elucidated. The multitude of available serological tests, based on diverse technologies (ELISA, CMIA, CLIA, ECLIA, LFIA, and Multiplex Immunoblot), highlights the need for evaluating the clinical performance of these methods. Factors such as the variability of targeted viral antigens, the detected antibodies (IgM, IgG, or both), disease severity, and

potential issues with cross-reactivity must be considered to ensure independent validation before their widespread use in clinical practice (2).

3.1.2. Results

The COVID-19 study group included 156 patients, predominantly male, with a median age of 50.5 years. Serological samples were collected at variable intervals from the onset of symptoms, with a median time from symptom onset to collection of 16 days. Hypertension, diabetes, and obesity were the most common comorbidities. Regarding disease severity, 39.7% of patients had mild forms, 33.9% had moderate forms, and 26.2% had severe forms. The sensitivity and specificity of IgG and IgM antibody tests varied depending on the method and the timing of sample collection. The IgG seropositivity rate gradually increased and remained above 95% for certain tests (CLIA and ECLIA) three months after symptom onset, although other tests (CMIA and ELISA) showed a slight decline in positivity rates over time. Concordance between IgG anti-N tests was high (up to 78%) but was low for IgM tests (19%).

3.1.3. Discussion

Compared to other studies, my research highlighted differences among commercial serological tests depending on the timing of symptom onset, with IgG persisting longer than IgM, which declined after four weeks, while the combined sensitivity of IgM and IgG was more effective than isolated detection of these antibodies. For instance, the sensitivity of ELISA IgG three weeks after symptom onset was similar to the results reported by Whitman, but the sensitivity of ELISA IgM in my study was lower (3). The lower sensitivity for samples in my study, especially beyond 15 days, can be explained by the low antibody titers frequently observed in patients with mild or asymptomatic forms of the disease, which predominated in the analyzed sample.

The CMIA IgG anti-N (Abbott), CLIA IgG anti-S1/S2 (DiaSorin), and ECLIA total anti-N (Roche) tests demonstrated comparable performance to those reported in other studies, but the overall sensitivity was influenced by the high proportion of patients with mild forms of the disease (4). Long-term evaluations indicate that anti-N and anti-S antibodies persist for at least six months (5), with the ECLIA total anti-N test showing high sensitivity and specificity for seroprevalence studies. In conclusion, test performance varies depending on disease severity

and the timing of sample collection, highlighting the need to evaluate multiple tests on diverse samples before their use in clinical practice.

3.1.4. Conclusion

The evaluated serological tests demonstrate high specificity; however, their sensitivity varies depending on the timing of symptom onset, disease severity, the method, and the antigen used. Therefore, the interpretation of serological results should be approached with caution and consider the clinical context. Combining IgM and IgG antibody testing could improve test performance, particularly for acute infections and cases with late presentation, atypical or prolonged symptoms, where RT-PCR may yield negative results.

3.2. Objective 2: Analysis of seroconversion and antibody dynamics in response to SARS-CoV-2 infection

3.2.1 Introduction

Seroconversion typically occurs between 10 and 21 days after the onset of infection, though this interval may vary depending on disease severity and individual immune response (6). In patients with mild forms of the disease, seroconversion may be delayed or even absent, whereas severe cases of COVID-19 are associated with a more intense and sustained immune response (7). This study analyzed the dynamics of seroconversion and the evolution of antibody concentrations using various serological methods, highlighting the importance of these data for understanding long-term immunity and optimizing diagnostic and treatment strategies.

3.2.2. Results

The study included 156 patients, the majority of whom were male (59.6%), with a median age of 50.5 years. Among them, 39.7% had mild forms, 33.9% moderate, and 26.2% severe forms of COVID-19. The rate of seroconversion and antibody detection varied between the serological tests, with CLIA IgG anti-S1/S2 and CMIA IgG anti-N showing the highest seroconversion rates (79.6%-87.5%), compared to ELISA IgM anti-N, which had a rate of 54.6%. Seroconversion occurred, on average, between 10 and 13 days after symptom onset, with differences depending on disease severity.

The analysis of antibody dynamics revealed that antibodies are detected earlier in patients with severe and moderate forms of the disease. After 90 days, patients with severe forms had higher seropositivity rates. Additionally, IgG antibody concentrations were significantly higher in patients with severe forms (202 AU/mL) compared to those with moderate and mild forms three months after symptom onset.

3.2.3. Discussion

An important aspect observed was the dynamics of IgM and IgG antibody appearance. In most cases, these antibodies were detected simultaneously; however, approximately one-third of patients presented IgM antibodies earlier than IgG. IgM antibody testing using the CMIA method (Abbott) demonstrated a higher positivity rate in the first few days after symptom onset (<5 days: 23%) and a sensitivity of 96% at three weeks, outperforming other methods such as ELISA. Nevertheless, IgG antibodies had a median seroconversion of 11-13 days, which aligns with the findings of other studies (8).

Regarding testing methods, performance varied depending on the target antigen. Tests targeting the N protein demonstrated faster and more sensitive detection compared to those targeting the S1/S2 protein (9). Differences between methods, such as CMIA, ELISA, and CLIA, can be attributed to the nature of the target antigens, their structure, and the cutoff values used. For instance, IgG antibodies detected using quantitative methods like CLIA enabled detailed evaluations of concentrations and the dynamics of the immune response.

Another determinant factor of the immune response was the severity of the disease. Patients with severe forms of COVID-19 exhibited higher antibody levels and faster seroconversion compared to those with moderate or mild forms (10). Ninety days after symptom onset, seropositivity rates were significantly higher in patients with severe forms, indicating a longer persistence of antibodies. Furthermore, quantitative antibody measurements revealed that patients with severe forms had significantly higher average levels than those with mild or moderate forms. This robust immune response may be linked to a higher viral load and severe systemic inflammation, which strongly stimulates the immune system.

Additionally, the variability of the immune response based on disease severity raises important questions regarding long-term protection against reinfection. Patients with mild forms, having lower antibody levels, might be more vulnerable to reinfection, while those with

severe forms could benefit from more prolonged protection. However, the durability of this response remains to be evaluated.

3.2.4. Conclusion

The study shows that most patients develop anti-SARS-CoV-2 antibodies, with the frequency and dynamics of seroconversion depending on the timing of sample collection and the testing methods used. IgG antibodies were more frequently detected than IgM, especially using ELISA. However, the CMIA test (Abbott) demonstrated a higher IgM positivity rate in the early days (83.8% within the first 10 days and 96% after three weeks). Simultaneous seroconversion of IgM and IgG was common, and in one-third of the patients, IgM appeared before IgG.

Tests based on the detection of the N protein exhibited higher sensitivity than those targeting the S protein, highlighting the importance of selecting the appropriate method for early diagnosis. Test performance varies depending on disease severity and the time since symptom onset, with ELISA tests being more sensitive to differences between mild and severe forms.

The differences in antibody titers between mild and severe forms underline the importance of post-COVID monitoring and the risks of reinfection. Quantitative measurements, such as CLIA IgG anti-S1/S2, are essential for assessing the immune response and guiding long-term treatment, including the need for booster doses in patients with mild forms.

4. Longitudinal analysis of the neutralizing capacity of anti-SARS-CoV-2 antibodies against the Delta variant and correlation with a commercial quantitative method for anti-S1/S2 IgG antibodies (DiaSorin)

4.1. Objective 3: Development of a variant of the neutralization technique using a surrogate marker (angiotensin-converting enzyme 2 receptor) and its correlation with one of the evaluated techniques, CLIA IgG (DiaSorin), a quantitative method for the detection of anti-S antibodies

4.1.1. Introduction

Humoral immunity, alongside innate immunity and the cellular immune response, plays a crucial role in managing SARS-CoV-2 infection. Although it is not yet clear whether

neutralizing antibodies (NAbs) are the primary mechanism of protection, they prevent the virus from entering cells and are essential for the development of long-term immunity. NAbs also play a significant role in assessing vaccine efficacy (11).

Although molecular detection techniques, such as RT-PCR, are valuable, there is an urgent need for reliable serological tests to monitor asymptomatic infections, fatality rates, and herd immunity. Currently, there are antibody tests that detect NAbs, but the most accessible ones, such as ELISA or rapid tests, cannot distinguish between neutralizing and non-neutralizing antibodies. A challenge remains in determining whether these simpler tests can provide accurate information about protective immunity (12).

The study proposes comparing a commercial quantitative method for assessing anti-spike S1/S2 IgG antibodies with a variant of a neutralization test that uses a surrogate marker to measure the ability of antibodies to block the interaction between the virus and host cells. This comparison aims to evaluate the accuracy and reliability of serological tests.

4.1.2. Results

The study included 25 patients, mostly young individuals with mild to moderate symptoms. Correlations between NAbs and anti-S1/S2 IgG antibodies were analyzed, revealing a weaker correlation between NAbs for the Delta variant and anti-S1/S2 IgG compared to the WT variant. Initially, the correlation was weak (r=0.39) but increased and remained consistently above 0.7 in subsequent visits. The correlation between NAbs for Delta and WT was consistently strong (above 0.76).

Regarding cross-neutralization (NT_{50}Delta/NT_{50}WT), a significant proportion of patients exhibited higher NT_{50} titers for the Delta variant compared to the WT variant at each of the three monitoring visits.

4.1.3. Discussion

Serological tests, such as CLIA IgG anti-S1/S2 (DiaSorin), demonstrated superior performance in diagnosing post-infection or post-vaccination immunity. However, neutralizing antibodies decline over time, which may lead to discrepancies between serological tests and neutralization titers.

According to the presented data, the humoral immune response can vary significantly, with serological tests such as DiaSorin S1/S2 demonstrating a relatively stable dynamic over time, unlike NT_{50} titers, which progressively declined for both the wild-type and Delta variants. Interestingly, in the studied group, a notable subset (24-28%) of participants exhibited more effective neutralization of the Delta variant compared to the WT variant, suggesting individual variability in the immune response. This observation is consistent with other studies (13, 14), which underscore the necessity of detailed monitoring of immunological responses to better understand their evolution.

The weak correlation observed between NT_{50} and the serological test at the first visit can be explained by the proximity of sample collection to the onset of symptoms, when the immune response is still developing. Additionally, cross-neutralization tests provide valuable information for guiding vaccine development strategies, particularly in the context of emerging viral variants.

4.1.5. Conclusion

The study highlighted strong correlations between neutralizing antibody titers for the Delta and WT variants, indicating a similar immune response. However, the more pronounced decline in antibodies for the Delta variant suggests differences in the efficacy of the immune response. Anti-S1/S2 IgG antibodies (DiaSorin) remained stable but did not reflect the decline in neutralization, limiting their utility as indicators of protection. Furthermore, the short-term stability of cross-neutralization indices underscores the limitations of the IgG (DiaSorin) test in assessing protection against viral variants.

4.2. Objective 4: Study of the neutralizing capacity of anti-SARS-CoV-2 antibodies from the initial period of the COVID 19 pandemic (wild-type strain) in comparison with subsequent viral variants (Delta)

4.2.1. Introduction

As the pandemic evolved, variants of concern (VOC) such as Alpha, Beta, Gamma, Delta, and Omicron emerged, featuring significant mutations that impact the efficacy of NAbs

and influence transmissibility and disease severity. The Delta variant, in particular, demonstrated high contagiousness and virulence, with reduced vaccine efficacy compared to other variants. Monitoring these variants is crucial for adapting vaccination and treatment strategies (15).

Longitudinal studies are essential for evaluating vaccine effectiveness and immune responses to emerging variants. Additionally, research on cross-neutralization provides insights into the ability of antibodies to combat multiple viral variants. The primary objective was to analyze the immune response to the original strain (WT) and subsequent variants (Delta), using measurements of IgG antibodies and neutralization titers.

4.2.2. Results

The study analyzed 25 participants with a median age of 51 years, the majority being women. Participants were monitored over three visits at intervals of 33, 90, and 195 days from the onset of symptoms. Most had mild and moderate forms of COVID 19, with 16% experiencing severe forms. The most common comorbidities were hypertension, cardiovascular diseases, and obesity.

Seropositivity for anti-S1/S2 IgG antibodies was 100% at the first visit, decreasing to 96% and 92% at visits 2 and 3, respectively. Regarding NAbs, all samples exhibited neutralization for both the WT and Delta variants at the first visit; however, the seropositivity rate declined significantly at visits 2 and 3 (88%-52% for WT and 60%-52% for Delta). NT_{50} titers showed a significant decline over time, more pronounced for the Delta variant (80%-84% decrease) compared to the WT variant (70%-77% decrease).

4.2.3. Discussion

Several studies have compared immune responses induced by vaccination and natural SARS-CoV-2 infection, highlighting differences in the effectiveness of protection (16). Some research suggests that unvaccinated individuals with prior infection have antibody concentrations equivalent to or even higher than those associated with 70% protection; however, only a small proportion reach levels that would ensure 90% protection (17). This indicates that vaccination can help achieve optimal antibody titers for durable protection.

In longitudinal studies, such as those conducted by Muecksch (13) and Moryiama (18), a significant reduction in neutralization efficiency against the Delta variant was observed, while vaccination significantly enhanced neutralization capacity. Similar research, like that of Kuzmina et al. (19), demonstrated a moderate decline in neutralization efficacy for the Delta variant but showed improved protection after vaccination.

4.2.4. Conclusion

The study highlights the variability of the immune response to SARS-CoV-2 infection, influenced by the severity and duration of the infection. The more pronounced decline in antibodies against the Delta variant suggests that protection may vary between variants. Continuous monitoring and the adjustment of vaccination and booster strategies are essential to maintaining protection.

5. Evaluation of risk factors associated with Long COVID (Objective 5)

5.1. Introduction

Long COVID, or chronic COVID syndrome, refers to a range of persistent symptoms that appear months after SARS-CoV-2 infection. Although there is no universally accepted definition, the World Health Organization defines long COVID as persistent symptoms occurring at least three months after the initial infection and lasting for a minimum of two months. Approximately 10-20% of infected individuals continue to experience long-term symptoms, such as extreme fatigue, breathing difficulties, cognitive issues, and pain (20).

Risk factors for long COVID include age, sex, comorbidities, severity of the infection, and viral load (21). Research on the relationship between antibody titers and long COVID has been contradictory. This study aimed to explore these associations and identify risk factors for the development of persistent symptoms.

5.2. Materials and methods for study 3

The study included adults diagnosed with SARS-CoV-2 infection, confirmed via RT-PCR, recruited in May 2020. Blood samples were collected at approximately 6+/-10 days, 33 +/-10 days (visit 1), and 91 +/-19 days (visit 2) from the onset of symptoms or diagnosis. Data

included demographic, clinical information, and persistent symptoms, with a telephone questionnaire applied to patients still presenting symptoms at the second visit to assess for long COVID.

Long COVID was defined as symptoms persisting beyond three months. Serological tests for anti-S1/S2 IgG antibodies were used to analyze the immune response, and statistical analyses compared patients with and without long COVID symptoms.

5.3. Result of study 3

The study initially included 93 patients, but 13 were excluded due to death or loss of contact, leaving a final analysis of 80 patients with a median age of 49.5 years. Of these, 44% were men, and 53.75% exhibited long COVID symptoms, most commonly fatigue, joint pain, memory problems, and breathing difficulties.

Women and patients with severe forms of the disease had a higher risk of developing long COVID, while comorbidities, particularly dyslipidemia, were associated with the presence of persistent symptoms. Treatments with steroids and Tocilizumab showed no significant association with long COVID. Additionally, patients with long COVID had higher levels of anti-S1/S2 IgG antibodies, but the differences were not statistically significant.

5.4. Discussion of study 3

Our study includes 80 patients with a balance between sexes and comorbidities, showing that fatigue is the most commonly reported symptom, similar to other studies (22), followed by dyspnea, cognitive impairment, and arthralgia. Compared to other studies (23), the prevalence of dyspnea in our cohort is lower, likely due to a smaller number of severe cases.

Regarding risk factors, we observed a significant association between female sex and the risk of long COVID, confirming findings from other studies reporting similar data (24). Our study did not identify age as a significant risk factor for developing long COVID, and the severity of the disease during the acute phase does not seem to impact the occurrence of persistent symptoms. These results align with other research (21, 25) that found no significant link between age or acute infection severity and long COVID.

Comorbidities, such as dyslipidemia, were associated with a higher risk of long COVID in our cohort.

The immune response, measured by anti-S1/S2 IgG antibodies, was not significantly correlated with the occurrence of persistent symptoms. These findings are consistent with other research (26) suggesting that the immune response alone is insufficient to predict long COVID. However, some studies suggest a link between a stronger humoral response and the risk of long COVID (27), warranting further investigation into this relationship.

The limitations of the study include the small sample size and the use of a single serological method, which may limit the generalizability of the results. Long-term monitoring is necessary to fully understand the duration and severity of long COVID symptoms.

5.5. Conclusion of study 3

The study highlighted that female sex and pre-existing comorbidities are significant risk factors for developing long COVID. In contrast, age, initial disease severity, immunomodulatory treatments, and anti-S1/S2 IgG antibodies were not significantly associated with the occurrence of symptoms. Although not all risk factors were identified, the analysis of antibody dynamics over different time periods provides valuable insights into their potential role in the development of long COVID.

Selective bibliography

1. Acuti Martellucci C, Flacco ME, Cappadona R, Bravi F, Mantovani L, Manzoli L. SARS-CoV-2 pandemic: An overview. Advances in Biological Regulation. 2020;77:100736.
2. Theel ES, Harring J, Hilgart H, Granger D. Performance Characteristics of Four High-Throughput Immunoassays for Detection of IgG Antibodies against SARS-CoV-2. Journal of Clinical Microbiology. 2020;58(8).
3. Whitman JD, Hiatt J, Mowery CT, Shy BR, Yu R, Yamamoto TN, et al. Evaluation of SARS-CoV-2 serology assays reveals a range of test performance. Nature Biotechnology. 2020;38(10):1174-83.
4. Van Elslande J, Decru B, Jonckheere S, Van Wijngaerden E, Houben E, Vandecandelaere P, et al. Antibody response against SARS-CoV-2 spike protein and nucleoprotein evaluated by four automated immunoassays and three ELISAs. Clinical Microbiology and Infection. 2020;26(11):1557.e1-.e7.
5. Favresse J, Eucher C, Elsen M, Gillot C, Van Eeckhoudt S, Dogné J-M, et al. Persistence of Anti-SARS-CoV-2 Antibodies Depends on the Analytical Kit: A Report for Up to 10 Months after Infection. Microorganisms. 2021;9(3):556.
6. Havers FP, Reed C, Lim T, Montgomery JM, Klena JD, Hall AJ, et al. Seroprevalence of Antibodies to SARS-CoV-2 in 10 Sites in the United States, March 23-May 12, 2020. JAMA Internal Medicine. 2020;180(12):1576.
7. Zhang X, Lu S, Li H, Wang Y, Lu Z, Liu Z, et al. Viral and Antibody Kinetics of COVID-19 Patients with Different Disease Severities in Acute and Convalescent Phases: A 6-Month Follow-Up Study. Virologica Sinica. 2020;35(6):820-9.
8. Ng DL, Goldgof GM, Shy BR, Levine AG, Balcerek J, Bapat SP, et al. SARS-CoV-2 seroprevalence and neutralizing activity in donor and patient blood from the San Francisco Bay Area. 2020.
9. Arkhipova-Jenkins I, Helfand M, Armstrong C, Gean E, Anderson J, Paynter RA, et al. Antibody Response After SARS-CoV-2 Infection and Implications for Immunity. Annals of Internal Medicine. 2021;174(6):811-21.
10. Brochot E, Demey B, Touzé A, Belouzard S, Dubuisson J, Schmit J-L, et al. Anti-spike, Anti-nucleocapsid and Neutralizing Antibodies in SARS-CoV-2 Inpatients and Asymptomatic Individuals. Frontiers in Microbiology. 2020;11.

11. Pang NY-L, Pang AS-R, Chow VT, Wang D-Y. Understanding neutralising antibodies against SARS-CoV-2 and their implications in clinical practice. Military Medical Research. 2021;8(1).

12. Tan CW, Chia WN, Qin X, Liu P, Chen MIC, Tiu C, et al. A SARS-CoV-2 surrogate virus neutralization test based on antibody-mediated blockage of ACE2–spike protein–protein interaction. Nature Biotechnology. 2020;38(9):1073-8.

13. Muecksch F, Wise H, Templeton K, Batchelor B, Squires M, McCance K, et al. Longitudinal variation in SARS-CoV-2 antibody levels and emergence of viral variants: implications for the ability of serological assays to predict immunity. 2021.

14. Takheaw N, Liwsrisakun C, Chaiwong W, Laopajon W, Pata S, Inchai J, et al. Correlation Analysis of Anti-SARS-CoV-2 RBD IgG and Neutralizing Antibody against SARS-CoV-2 Omicron Variants after Vaccination. Diagnostics. 2022;12(6):1315.

15. ECDC. Variants of concern: ECDC; 2024 [updated July 2024]. Available from: https://www.ecdc.europa.eu/en/covid-19/variants-concern.

16. Feng S, Phillips DJ, White T, Sayal H, Aley PK, Bibi S, et al. Correlates of protection against symptomatic and asymptomatic SARS-CoV-2 infection. Nature Medicine. 2021;27(11):2032-40.

17. Schuh AJ, Satheshkumar PS, Dietz S, Bull-Otterson L, Charles M, Edens C, et al. SARS-CoV-2 Convalescent Sera Binding and Neutralizing Antibody Concentrations Compared with COVID-19 Vaccine Efficacy Estimates against Symptomatic Infection. Microbiology Spectrum. 2022;10(4).

18. Moriyama S, Adachi Y, Sato T, Tonouchi K, Sun L, Fukushi S, et al. Temporal maturation of neutralizing antibodies in COVID-19 convalescent individuals improves potency and breadth to circulating SARS-CoV-2 variants. Immunity. 2021;54(8):1841-52.e4.

19. Kuzmina A, Wattad S, Khalaila Y, Ottolenghi A, Rosental B, Engel S, et al. SARS CoV-2 Delta variant exhibits enhanced infectivity and a minor decrease in neutralization sensitivity to convalescent or post-vaccination sera. iScience. 2021;24(12):103467.

20. WHO. Post COVID-19 condition (Long COVID): WHO; 2024 [updated April 2024. Available from: https://www.who.int/europe/news-room/fact-sheets/item/post-covid-19-condition.

21. Yong SJ. Long COVID or post-COVID-19 syndrome: putative pathophysiology, risk factors, and treatments. Infectious Diseases. 2021;53(10):737-54.
22. Liang L, Yang B, Jiang N, Fu W, He X, Zhou Y, et al. Three-Month Follow-Up Study of Survivors of Coronavirus Disease 2019 after Discharge. Journal of Korean Medical Science. 2020;35(47).
23. Garrigues E, Janvier P, Kherabi Y, Le Bot A, Hamon A, Gouze H, et al. Post-discharge persistent symptoms and health-related quality of life after hospitalization for COVID-19. Journal of Infection. 2020;81(6):e4-e6.
24. Notarte KI, de Oliveira MHS, Peligro PJ, Velasco JV, Macaranas I, Ver AT, et al. Age, Sex and Previous Comorbidities as Risk Factors Not Associated with SARS-CoV-2 Infection for Long COVID-19: A Systematic Review and Meta-Analysis. J Clin Med. 2022;11, 7314.
25. Peghin M, Palese A, Venturini M, De Martino M, Gerussi V, Graziano E, et al. Post-COVID-19 symptoms 6 months after acute infection among hospitalized and non-hospitalized patients. Clinical Microbiology and Infection. 2021;27(10):1507-13.
26. Rank A, Tzortzini A, Kling E, Schmid C, Claus R, Löll E, et al. One Year after Mild COVID-19: The Majority of Patients Maintain Specific Immunity, But One in Four Still Suffer from Long-Term Symptoms. Journal of Clinical Medicine. 2021;10(15):3305.
27. Klein J, Wood J, Jaycox J, Lu P, Dhodapkar RM, Gehlhausen JR, et al. Distinguishing features of Long COVID identified through immune profiling. 2022.

The list of publications related to the doctoral thesis topic

Articles published in full

1. **Nedelcu I**, Jipa R, Vasilescu R, Baicus C, Popescu C, Manea E, Stoichitoiu L, Pinte L, Damalan A, Simulescu O, Stoica I, Stoica M, Hristea A, *Long-term longitudinal evaluation of six commercial immuno-assays for the detection of IgM and IgG antibodies against SARS CoV-2*, Viruses; 2021, 13(7), 1244, FI - 5,81/2021, Q2, Chapter II of doctoral thesis, pag. 45-47, 52-75
   https://www.mdpi.com/1999-4915/13/7/1244

2. **Nedelcu I**, Florian P, Ion D, Militaru E, Damalan A, Popescu C, Hristea A, *Dynamics of serum cross-neutralization capacity against SARS-CoV-2 Delta variant in convalescent COVID-19 patients*, Journal of Medical Virology, 2024, 96:e29448. 9, FI – 6,8/2023(2024 update), Q1, Chapter II of doctoral thesis, pag. 48-51, 76-97
   https://doi.org/10.1002/jmv.29448

3. **Nedelcu I**, Militaru E, Damalan A, Băicuș C, Marin L, Hristea A, *Risk factors and antibody response associated with long COVID: longitudinal cohort study*, The Medical-Surgical Journal, 2024, 128(3), 490-498, FI – 0,1/2023, Q4, Chapter II of doctoral thesis, pag. 98-111
   https://www.revmedchir.ro/index.php/revmedchir/article/view/3009

Abstracts published and presented at national conferences

1. **Nedelcu I**, Damalan A, Vasilescu R, Jipa R, Manea E, Hristea A, *Validation of two serology assays commercially available in COVID 19 diagnosis*, The 13th National Congress of Infectious Disease COVID 19, Cluj-Napoca, Romania, 17-18 Oct 2020, Chapter II of doctoral thesis, pag45-47, 52-75. Abstract accepted as oral presentation.

2. **Nedelcu I**, Damalan A, Vasilescu R, Jipa R, Manea E, Hristea A, *Factors influencing the production of IgG SARS-CoV-2 antibodies*, 16th Edition of the Scientific Days of the National Institute for Infectious Diseases"Prof Dr Matei Bals", Bucharest, 26- 30 Oct, 2020, summary volume pag 6-7, Chapter II of doctoral thesis, pag. 45-47, 52-75. Abstract accepted as oral presentation. Publication indexed: ISSN 2457-8525

3. **Nedelcu I**, Neagu G, Vasilescu R, Jipa R, Manea E, Damalan A, Hristea A, *Influencing factors for anti SARS CoV-2 S1/S2 antibodies production determinated by a quantitative assay*, National Conference of Infectious Pathology, Iasi, 24-26 June, 2021, Chapter II of doctoral thesis, pag. 45-47, 52-75. Abstract accepted as oral presentation.

4. **Nedelcu I**, Militaru E, Damalan A, Patrascu R, Hristea A, *Assessment of anti-SARS-CoV-2 antibodies using commercially available methods*, The 14th National Congress of Infectious Disease, Iasi, 20-22 Oct, 2022, Volume of summaries; 385-386, Chapter II of doctoral thesis, pag. 45-47, 52-75. Abstract accepted as oral presentation. Publication indexed ISBN 978-606-544-773-8

5. Popescu C, **Nedelcu I**, Florian P, Ion D, Militaru E, Damalan A, Costache A, Ionescu I, Onu A, Hristea A, *Dynamics of SARS CoV-2 cross-neutralizing antibody response in convalescent Covid-19 patients – molecular mechanisms involved in SARS CoV-2 seroneutralization*, HIV Sibiu Dialogues VI, 06-08 June, 2024, Chapter II of doctoral thesis, pag. 48-51, 76-97. Abstract accepted as oral presentation.