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Effects of the Third Dose of the Coronavirus Disease Vaccine in Healthy Adults and Immunosuppressed Patients

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Abstract

This study evaluated the antibody and cellular immune responses before and after the third dose of the coronavirus disease 2019 (COVID-19) vaccine in healthy individuals and immunocompromised patients. At baseline, the healthy individuals exhibited 100%

seropositivity rate with significantly higher antibody titers compared to immunocompromised patients, who exhibited a lower seropositivity rate (>90%) and markedly reduced antibody levels. Among the immunocompromised subgroups, kidney transplantation (KT) recipients had the lowest seropositivity rate (73.3%) and significantly lower antibody titers than patients with rheumatoid arthritis, whose responses were comparable to those of the healthy individuals. Following the third vaccine dose, all groups demonstrated an increased seropositivity rate; however, the KT patients continued to exhibit significantly lower antibody titers and interferon (IFN)-γ levels. At 6 months post-vaccination, approximately 93.2% of the KT recipients remained seropositive; however, their median antibody titers and neutralizing activity remained significantly lower than those of the other groups. T-cell responses were also substantially reduced in the immunocompromised individuals, particularly in the KT recipients, who consistently exhibited the lowest IFN-γ levels throughout the observation period. Among all groups and time points, neutralizing antibodies against the Omicron variant were lower than those against the wild-type virus, with the KT recipients demonstrating almost zero neutralizing activity against Omicron, even after receiving the booster dose. Although the third dose improved the immune responses of most of the immunocompromised subgroups, the KT recipients consistently exhibited weak antibody and T-cell responses, especially against Omicron, even after receiving the booster dose. The correlation between antibodies and the IFN-γ release assay was weak and nonsignificant in the healthy individuals, whereas it was strong and significant in the immunocompromised patients. Furthermore, the baseline immune parameters were more predictive of post-vaccination responses in the high-risk individuals than they were in the healthy controls.

Keywords: COVID-19; SARS-CoV-2; Vaccination

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a double-stranded ribonucleic acid (RNA) virus that has a length of approximately 30,000 nucleotides. It comprises the spike (S) protein, envelope, membrane, and nucleocapsid and enters the host cell by binding between the S protein and angiotensin-converting enzyme 2 (ACE2) receptor [1].

The primary coronavirus disease 2019 (COVID-19) vaccines in South Korea are viral vector vaccines (AstraZeneca and Janssen) and messenger RNA (mRNA) vaccines (Moderna and Pfizer) [2,3]. Currently, the reported mortality rate among immunosuppressed solid organ transplant recipients is 20% [4]. Moreover, the seroconversion rate at 1 month after receiving the second dose of mRNA vaccines in solid organ transplant recipients is low (124/367 persons; 33.8%). Therefore, increasing the vaccine dose or administering a third dose may be considered for this population [5].

To determine the effects of the third COVID-19 vaccine dose in healthy adults and patients with immunosuppression, including hematologic malignancy, solid cancer, rheumatoid arthritis, and renal transplant patients, we compared the S protein-binding antibody titer, interferon-γ (IFN-γ) levels, plaque reduction neutralization test $(PRNT)_{50}$ titer, and surrogate virus neutralization test (sVNT) using a rapid kit before vaccination and at 1, 3, and 6 months after vaccination.

2. Methods

Study design and patient population

This study enrolled healthy adults and immunosuppressed patients, including those with hematological malignancies, solid cancer, or rheumatoid arthritis and renal transplant recipients. We also collected data on patient characteristics, including sex, age, and type of vaccine received. Blood samples were collected before and at 1, 3, and 6 months after the third vaccination. All immune response analyses were performed at a single central laboratory by the same trained investigator using patient samples collected from multiple institutions. Each individual was vaccinated according to the government policies regarding age and vaccination type. The methodology of this study followed the relevant guidelines and regulations stated in the Methods section.

The inclusion criteria for the patients with solid or blood cancer were an age of ≥19 y, a waiting period of 3 months ± 4 weeks after receiving the second COVID-19 vaccine dose, a daily activity performance score of 0–2 on the European Cooperative Oncology Group scale, a waiting period of 3 months after hematopoietic stem cell transplantation, and a life expectancy of ≥12 months. The inclusion criteria for the patients with rheumatoid arthritis were an age of ≥19 y, a diagnosis with rheumatoid arthritis, a waiting period of 3–4 weeks after receiving the second COVID-19 vaccine dose, and a stable administration of an antiarthritic drug in the past 3 months. The inclusion criteria for the renal transplant recipients were an age of ≥19 y and a waiting period of 3 months ± 4 weeks after receiving the second COVID-19 vaccine dose. The inclusion criteria for the healthy adults were an age of ≥19 y and the absence of a COVID-19 infection. Written informed consent was obtained from all the participants. The exclusion criteria were severe allergic reaction or thrombosis after receiving the COVID-19 vaccine, suspected COVID-19 infection, untreated severe infectious diseases, suppressed bone marrow (neutrophil count < 500/mm^3 or platelet count < 50,000/mm^3), local hemostatic abnormalities, and intravenous immunoglobulin administration within the past 28 d. This multicenter observational study was conducted at Chosun University Hospital, with Chonnam National University Hospital, Chonnam National University Hwasun Hospital, Asan Medical Center, and Samsung Medical Center participating as collaborating institutions. This study was conducted with the approval of the institutional review board of each center. The experimental methods and statistical analyses are described in the supplementary files.

Binding antibody analysis via electrochemiluminescence immunoassay

Elecsys® anti-SARS-CoV-2 (Roche Diagnostics, Mannheim, Germany) is a commercial electrochemiluminescence immunoassay used to measure the immunoglobulin G antibody titer against its reaction with the receptor-binding domain of the S protein of SARS-CoV-2, as previously described. It has a measuring range of 0.4–250 U/mL (up to 2500 U/mL with onboard 1:10 dilution and up to 12500 U/mL with onboard 1:50 dilution). Values higher than 0.8 U/mL were considered positive [6].

T-cell immune response analysis via IFN-γ release assay

To investigate the cellular immune responses in both healthy and immunocompromised patients after receiving the third dose of the anti-SARS-CoV-2 vaccine, an IFN-γ release assay (IGRA) was performed using an enzyme-linked immunosorbent assay (ELISA; CoviFERON ELISA, SD Biosensor, Suwon-si, Gyeonggi-do, South Korea). The IFN-γ levels induced by SARS-CoV-2 wild-type/alpha S protein (SP) and SARS-CoV-2 beta/gamma SP have been measured [7]. Based on the 0.25 U/mL cutoff recommended by the manufacturer, the participants were categorized into positive and negative groups, and the risk factors were analyzed by comparing the participants' characteristics. All immune response analyses were performed at a single central laboratory by the same trained investigator using patient samples collected from multiple institutions.

Neutralizing antibody assay

Neutralizing antibody assay to assess the neutralizing antibody titer against SARS-CoV-2 in both healthy and immunocommpromised patients after receiving three doses of the vaccine, PRNT was performed using Vero E6 cells (*Cercopithecus aethiops* kidney epithelial cells, CRL-1586, ATCC). The neutralizing antibody titer was measured against the SARS-CoV-2 wild-type (V clade [B lineage], isolated hCoV-19/South Korea/KUMC01/2020, GISAID accession no. EPI_ISL_413017) and Omicron variant (GR clade [B.1.1.529 lineage]), and isolated hCoV-19/Korea/KDCA447321/2021 variant (accession no. NCCP43408) was purchased from the Korea Disease Control and Prevention Agency. The $PRNT_{50}$, the level at which SARS-CoV-2 proliferation is suppressed by 50%, was defined as the neutralizing antibody titer [8]. The participants were categorized into positive and negative groups using a cutoff of 1:8, and the risk factors were analyzed by comparing their characteristics [9].

Neutralizing antibody titer analysis using the GenBody rapid kit

The GenBody fluorescence immunoassay (FIA) COVID-19 neutralizing antibody (NAb) kit (GenBody, Cheonan-si, Chungcheongnam-do, South Korea) was used to perform the surrogate virus neutralizing test (sVNT). The GenBody sVNT is a type of fluoroimmunoassay used to measure the inhibition of RBDACE2 binding based on the antibody-mediated blockage of the interaction between ACE2 and wild-type SARS-CoV-2 SP. Following the manufacturer's guideline, a cutoff of ≥30% (GenBody) was applied [10].

Statistical analysis

Demographic characteristics were compared between the healthy adults and immunocompromised group and among the subgroups of immunocompromised patients using the chi-square test for sex and vaccine type, while the median age (interquartile range) was compared between the healthy adults and immunocompromised group and among the subgroups of immunocompromised patients using analysis of variance. The Mann–Whitney U test was used to compare the S protein-binding antibody titers, IFN-γ levels, and PRNT NAb titers for the wild-type and Omicron SARS-CoV-2 variants between the healthy adults and immunocompromised patient group, as well as among the subgroups of immunocompromised patients, at each time phase, specifically before and at 1 month after the third vaccination. Spearman's rho was used to test the correlation between the PRNT titer and median percentage of inhibition using the sVNT and initial value, as well as the correlation between the humoral and cellular immune responses before the third vaccination and at 1, 3, and 6 months after the third vaccination. Fisher's exact test was used to compare the positivity rate of each test between the healthy and immunocompromised patients and among the immunocompromised subgroups. The data were analyzed using GraphPad Prism, version 8.0.1 (GraphPad Software, USA), and Statistical Product and Service Solutions, version 26 (IBM, USA).

3. Results

A total of 392 individuals were screened, among which 369 participants were recruited. Of these, two dropped out, resulting in 367 participants enrolling in the study. Among them, 144 were healthy, and 223 were immunocompromised (56 had hematologic malignancies, 75 had solid tumors, 47 had rheumatic diseases, and 45 were kidney transplant recipients). Due to there being shortage of sVNT kits, the analysis included only the participants who underwent testing using all three assays: the binding antibody assay, IGRA, and sVNT. The participants included 42 healthy individuals, 55 patients with hematological malignancies, 73 patients with solid cancers, 43 patients with rheumatoid arthritis, and 45 kidney transplant recipients.

Demographic characteristics

A significant difference in sex was observed between the healthy adult group and the immunocompromised group ($p = 0.002$) but not among the immunocompromised subgroups ($p = 0.235$). Furthermore, a significant difference ($p = 0.001$) was observed in the type of vaccine, with mRNA/mRNA/mRNA being the most commonly administered type. This trend was observed in the immunocompromised subgroups, except for the solid cancer group, in which the most common vaccine regimen was Ad/Ad/mRNA. 1. The mRNA-based vaccines include those manufactured by Pfizer-BioNTech (BNT162b2/Comirnaty) and Moderna (mRNA-1273/Spikevax). Viral vector vaccines (nonreplicating) include those manufactured by AstraZeneca (AZD1222/Vaxzevria) and Janssen (Johnson & Johnson/Ad26.COV2.S). Additionally, the median age of the immunocompromised group was greater than that of the healthy adults (Table 1).

Binding antibody analysis via double-antigen indirect sandwich enzyme-linked immunosorbent assay

The antibody responses after receiving the third COVID-19 vaccine dose were consistently lower in the immunocompromised patients than in the healthy adults, as shown in supplementary table 1 and figure 1. Before receiving the third dose, 100% of the healthy adults were seropositive, compared with 93.1% of immunocompromised patients; the kidney transplant recipients had the lowest seropositivity rate (73.3%) and median titer (8.5 U/mL, $p < 0.001$). At 1 month post-vaccination, the seropositivity increased to above 95% in most groups, except for the kidney transplant recipients (82.2%), who had persistently low titers (566 U/mL, $p < 0.001$). At 3 and 6 months post-vaccination, the seropositivity rate remained high, except for the kidney transplant recipients, who demonstrated lower rates and titers throughout the follow-up period (median 791.6 and 2229.5 U/mL, respectively; $p < 0.001$ and $p = 0.027$, respectively). The correlation between baseline S antibodies and the response was weak in the healthy adults at 1 month post-vaccination (r = 0.03), but it was stronger at 3 and 6 months (r = 0.75 and r = 0.67, respectively). The immunocompromised patients demonstrated significant and consistent correlations for all time points (r = 0.68–0.38, $p < 0.0001$), and the strongest correlations were observed in the kidney transplant recipients (up to r = 0.77). This suggests that baseline antibody levels reliably predict the vaccine response in immunocompromised individuals, especially in kidney transplant patients. This can be seen in Supplementary Table 4.

T-cell immune response analysis via IFN-γ release assay

S-specific IFN-γ responses were significantly lower in the immunocompromised patients than in the healthy adults at all time points, as shown in supplementary table 2 and figure 2. Before receiving the third vaccine dose, the T-cell responses to both wild-type and variant S proteins were especially low in the immunocompromised groups, with the kidney transplant recipients exhibiting the weakest seropositivity rate (≤9%). At 1 month post-booster, the IFN-γ levels increased in all of the groups but remained significantly lower in the immunocompromised patients (median of 0.52 IU/mL vs. 2.14 IU/mL; $p < 0.001$), with the kidney transplant recipients exhibiting the lowest response (0.13 IU/mL). At 3 and 6 months, the IFN-γ level declined across all of the groups but remained high in the healthy individuals. Among the immunocompromised patients, the patients with rheumatoid arthritis and patients with solid cancer had relatively better responses, whereas the transplant recipients exhibited persistently low IFN-γ levels (6-month median: 0.34 IU/mL).

The correlation patterns between the baseline IFN-γ level and post-vaccine response differed among the groups. The healthy individuals demonstrated a higher and significant correlation for the S variant (r = 0.63–0.81, p = 0.001) than for the original S, which had moderate, sometimes nonsignificant correlations. In the immunocompromised patients, the correlations were more stable and significant mainly for the original S (r = 0.33–0.43; $p < 0.0001$). The patients with hematological cancer and those with solid cancer had consistent correlations for both S types; the rheumatoid arthritis patients' correlation improved at 3 months (r = 0.48; $p = 0.007$). The kidney transplant patients demonstrated weak, nonsignificant correlations, especially at 6 months (r < 0.3), reflecting their poor cellular immunity. Overall, the baseline IFN-γ level can predict the post-vaccine cellular immune response, but its reliability depends on the immune status of the individual and underlying disease, as shown in Supplementary table 4.

Neutralizing antibody titers

The neutralizing antibody responses to the wild-type virus were consistently higher than those to the omicron virus across all groups and time points, as shown in table 2 and figure 3. Before receiving the third vaccine dose, most of the immunocompromised patients had a detectable level of neutralization against the wild-type virus (73.3%) but a much lower level against the Omicron variant (24.4%), with the kidney transplant recipients showing no neutralization. At 1 month post-booster, the titers increased for all of the groups but remained lower against the Omicron variant, especially in the kidney transplant patients (median of 1:1 vs. 1:64–1:1024 in the others). Only the patients with rheumatoid arthritis and solid tumors demonstrated a significant increase. At 3 and 6 months, the Omicron neutralization declined faster than the wild-type, particularly in the immunocompromised individuals, with the kidney transplant recipients consistently demonstrating the lowest decline. The correlation between the baseline neutralizing antibody response (PRNT) and post-vaccine response varied: the healthy individuals exhibited a fairly strong but nonsignificant correlation against the wild-type virus at 6 months (r = 0.71), while the immunocompromised patients had significant correlations over time (r = 0.49–0.62; p < 0.05). The patients with rheumatoid arthritis had the strongest correlation with the wild-type virus (r = 0.88; p = 0.002), whereas the cancer and transplant patients demonstrated weaker correlations. For the Omicron variant, the correlations were generally weaker; only the immunocompromised patients demonstrated a significant correlation at 1 month (r = 0.52; p = 0.0004), which declined by 6 months. The strongest correlation was demonstrated in the patients with rheumatoid arthritis (r = 0.72; p = 0.027). Overall, the baseline antibody levels predicted responses to the wild type virus better than they did to the Omicron variant, especially in the immunocompromised groups These findings are presented in Supplementary Table 4.

Neutralizing antibody titer analysis using the GenBody rapid kit

Before the third vaccination, the healthy individuals had a significantly higher seropositivity rate (97.56%) than the immunocompromised patients (62.5%) (p < 0.0001). Among the immunocompromised subgroups, the seropositivity rates varied as follows: hematologic malignancy (72.73%), solid cancer (61.64%), rheumatoid arthritis (86.05%), and kidney transplantation (28.89%) (p < 0.0001), as shown in supplementary table 3 and figure 4. The median antibody titers were the highest in the healthy individuals (90.34 U/mL) and lowest in the kidney transplant recipients (13.15 U/mL; p < 0.001). At 1 month post-vaccination, seropositivity rate increased to 100% in healthy and 87.79% in immunocompromised patients (p = 0.0854), with the patients with rheumatoid arthritis exhibiting the highest response (100 %), and the kidney transplant recipients exhibiting the lowest response (64.44 %) (p < 0.0001). The titers remained the highest in the healthy individuals (98.67 U/mL) and lowest in the the kidney transplant group (64.92 U/mL; *p* < 0.001). By 3 months, the seropositivity of the healthy individuals reached 100% and that of the immunocompromised patients reached 92.86% (p = 0.3709), and there was significant variation among the subgroups (p < 0.0075). The lowest titers were again seen in the kidney transplant recipients (81.36 U/mL), and the highest titers were exhibited in the patients with rheumatoid arthritis (98.88 U/mL; p < 0.001). Neutralizing activity assessed by the sVNT demonstrated no significant changes in the healthy individuals from baseline to 1 month (r = 0.36, p = 0.118) or 3 months (r = 0.28, p = 0.232). In contrast, the immunocompromised patients demonstrated significant increases at both time points (r = 0.49 and r = 0.53; p < 0.0001). Within the subgroups, solid cancer and kidney transplant recipients demonstrated consistent and significant improvements (r = 0.54 and 0.59, respectively). Patients with hematologic malignancies demonstrated a delayed significance, whereas the patients with rheumatoid arthritis exhibited no significant change in sVNT inhibition. The correlations supporting these findings are presented in Supplementary Table 4.

The relationship between T-lymphocyte and humoral responses

In healthy individuals, before vaccination, the correlation between antibodies and IGRA was low and nonsignificant; however, after vaccination, the correlation began to increase, although it was not always significant, especially within 1–3 months. In immunocompromised patients and those with various serious medical conditions (such as hematologic cancer, solid cancer, rheumatoid arthritis, and kidney transplantation), the correlation between S protein-binding antibodies and the IGRA response was stronger and significant, particularly after vaccination. This correlation tended to persist for up to 6 months after vaccination in the immunocompromised group, indicating that the humoral immune response (antibodies) and cellular immune response (IGRA) remain closely related over a relatively long period in this group. These findings are presented in Supplementary Table 5.

4. Discussion

The Centers for Disease Control and Prevention notes that, although vaccine effectiveness declines over time, it still protects against severe illness and death [11]. Although vaccine responses have been studied in healthy and immunocompromised individuals [12,13], direct comparisons between the two groups are limited.

In this study, the immunocompromised patients achieved seropositivity after the third dose. However, their antibody levels, especially those in the kidney transplant recipients, remained significantly lower than those in the healthy adults. These findings are consistent with those of Mazolla et al., who reported a lower seroconversion rate in solid organ transplant recipients (28.6%) than in healthcare workers (100%) ($p < 0.0001$), with kidney transplant recipients demonstrating the lowest rate (16.6%) [14]. Marion et. demonstrated that seropositivity in solid organ transplant recipients increased from 1.4% to 33.8% after receiving a second vaccine dose [5]. Consistent with this, Pedersen et al. reported an undetectable S-specific antibody in kidney transplant patients at 4 weeks post-vaccination [15].

In the study by Monin et al., after receiving two doses of the BNT162b2 (Pfizer®) vaccine, the seroconversion rate of healthy adults was 100%, that of patients with solid cancers was 95%, and that of patients with hematologic cancers was 60%, with much lower neutralizing activity in the third group [16]. Similarly, our study demonstrated that, at 1 month after the third dose, the seropositivity rate reached 100% for patients with solid cancers and 98.2% for patients with hematologic malignancies; however, the antibody titers remained significantly lower in the latter group ($p < 0.001$). These results indicate that, although a third vaccine dose boosts immune responses, patients with hematologic malignancies consistently exhibit weaker responses than those with solid tumors.

Fernández-Ruiz et al. demonstrated lower IFN-γ levels in liver transplant recipients than in healthy adults [15], and Pedersen et al. reported no detectable IFN-γ in kidney transplant patients after receiving a second vaccine dose. In this study, the IFN-γ levels were also assessed before and at 1 month after the third dose, with the highest levels observed in healthy adults and the lowest in kidney transplant recipients (supplementary table S2). Similarly, Monin et. al. reported reduced T-cell responses in cancer patients after receiving two vaccine doses, with stronger responses in those with solid tumors than in those with hematologic malignancies [5]. Consistent with these findings, immunocompromised patients, particularly those with blood cancers and kidney transplants, had significantly lower IFN-γ levels than healthy adults before receiving a third vaccine dose. Although IFN-γ levels increased post-booster in all groups, patients with solid tumors maintained stronger responses, while those with blood cancers and kidney transplants consistently exhibited the weakest responses over the 3–6-month follow-up period.

This study confirmed a low Nab response in immunocompromised patients, particularly in kidney transplant recipients. Pedersen et. al. demonstrated that PRNT titers in this patient group remained below 1:4 at post-vaccination, indicating no NAb response [16]. Similarly, prior to receiving a third vaccine dose, NAbs against the wild-type virus were detected in 73.3% of immunocompromised patients, but only 24.4% responded to the Omicron variant, with none detected in the kidney transplant recipients. At 1 month post-booster, the titers increased but remained the lowest in this group (median: 1:1 vs. 1:64–1:1024 in the others). In contrast, the patients with rheumatoid arthritis and solid cancers exhibited a stronger response. Monin et al. also reported higher seroconversion rates and NAb activity in patients with solid cancers than in those with hematological malignancies after receiving a second vaccine dose [17]. Our study supports this finding, demonstrating that patients with solid cancers maintained higher antibody titers and neutralization levels than did blood cancer patients for up to 6 months. The NAb activity remained more stable against the wild-type strain, whereas responses to the Omicron variant declined rapidly in the immunocompromised groups. Although the third vaccine dose improved immunity across all groups, their responses varied. The kidney transplant recipients consistently exhibited the weakest immunity, whereas the patients with solid cancers and those with rheumatoid arthritis demonstrated a stronger level of immunity, underscoring the need for tailored vaccination strategies. Indirect testing of Nabs using SVNT is limited to the wild-type

S protein and does not assess responses to newer variants, such as Omicron BA.4 or BA.5 [18,19]. Therefore, variant-specific sVNTs are needed as these strains continue to circulate, even among vaccinated individuals [21].

The Advisory Committee on Immunization Practices recommends the first and third doses at a 28-d interval in immunosuppressed patients [20]. After two doses, seronegativity rates in transplant recipients remained high (50–70%), but seroconversion improved after a third dose, with side effects similar to earlier doses. This study also demonstrated enhanced immunogenicity following a third vaccination in both the healthy and immunosuppressed individuals. However, the responses in patients such as kidney transplant recipients remain suboptimal, supporting the need for continued precautions and possibly additional vaccine doses.

This study has several limitations. First, $PRNT_{50}$ titers were not available for all patients, and sVNT based on the wild-type S protein could not assess the neutralization of Omicron. Second, the IGRA kit used only wild-type and early variant antigens, limiting the evaluation of T-cell responses to the Omicron variant. Third, as this was an observational study, the participants were not randomized according to age, vaccine type, or prior COVID-19 infection status, But the correlation between PRNT titer and inhibition rate from several recovered patients can be seen in Supplementary Figures 1, 2 and 3, and Supplementary Tables 6 and 7. Fourth, we lacked comparative data to assess whether the third dose provided sufficient protection in the immunocompromised individuals, such as transplant recipients, compared with the general population after two doses. Finally, owing to the limited sample size, the responses could not be compared across different types of immunocompromised patients. In conclusion, both the humoral and cellular immune responses improved at 1 month after receiving the third vaccine dose in all of the groups, but the increase was significantly lower in immunocompromised patients, especially in the kidney transplant recipients.

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Author contributions

Concept and design: D. M. K.; acquisition, analysis, or interpretation of data: S. K., M.-S. B., D. Y. K., J.-W. S., N. R. Y., C.-M. K., J.-H. K., J. Y., K. R. P., K. W. L., S.-H. J., H.-J. B., W. K. B., T.-J. K., K. H. B., S.-H. K., J.-H. L., and M. M.; drafting of the manuscript: S. K.,D. M. K., and M. M.; critical revision of the manuscript: M. M., M.-S. B., and D. M. K.; administrative, technical, or material support: S.-H. J. and W. K. B.; supervision: D. M. K. and W. K. B. All authors have read and agreed to the published version of the manuscript.

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*** Ethical approval**

Approval for this study was granted by the institutional review board at Chosun University Hospital (approval number: 2021-10-018).

Declaration of interest

The authors declare that they have no competing interests.

Data availability:

All data generated or analyzed in this study are included in the published article [and supplementary information files].

Figure legends

Figure 1. Anti-spike protein antibody responses to a third vaccination in immunosuppressed patients and healthy adults. A. Before the third vaccination; B. 1 month after the third vaccination; C. 3 months after the third vaccination; D. 6 months after the third vaccination.

Figure 2. Spike-specific T-cell responses to a third vaccination in immunosuppressed patients and healthy adults. A. Before the third vaccination; B. 1 month after the third vaccination; C. 3 months after the third vaccination; D. 6 months after the third vaccination.

Figure 3. Plaque reduction neutralization test-neutralizing antibody titers to a third vaccination in immunocompromised patients and healthy adults. A. Before the third vaccination; B. 1 month after the third vaccination; C. 3 months after the third vaccination; D. 6 months after the third vaccination.

Figure 4. Surrogate virus neutralization test-neutralizing antibody titers to a third vaccination in immunocompromised patients and healthy adults. A. Before the third vaccination; B. 1 month after the third vaccination; C. 3 months after the third vaccination; D. 6 months after the third vaccination.

Tables

Table 1. Demographic characteristics of the enrolled healthy and immunocompromised patients

Characteristics	Healthy (a) (n = 42)	Immunocompromised (n = 216)	*p*-value	Hematologic malignancy (b) (n = 55)	Solid cancer (c) (n = 73)	Rheumatoid arthritis (d) (n = 43)	Kidney transplantation (e) (n = 45)	*p*-value
Male gender, N (%)	21 (50.0%)	159 (61.6%)	0.002*	32 (58.2%)	52 (71.2%)	26 (60.5%)	28 (62.2%)	0.235*
Age, Median, IQR	34.5 (28.75–41.5)	58.0 (49.25–67.0)	< 0.001**	60.0 (55.0–71.0)	64.0 (58.0–71.5)	51.0 (30.0–59.0)	49.0 (38.0–54.5)	< 0.001***
Vaccination type								
Ad/Ad/mRNA N (%)	1 (2.4%)	75 (29.1%)		19 (25.3%)	45 (60.0%)	4 (5.3%)	6 (8%)	
mRNA/mRNA/mRNA, N (%)	29 (69.0%)	152 (58.9%)	< 0.001*	33 (60%)	26 (35.6%)	39 (90.7%)	25 (55.6%)	<0.001*
Other group N (%)	12 (28.6%)	31 (12%)		3 (9.7%)	2 (6.5%)	0 (0%)	14 (31.1%)	

* The Chi-square test or Fisher's exact test and ** the Mann–Whitney U test were used to perform comparisons between the healthy adults and patients with immunosuppression.
*** The Kruskal–Wallis test was used to perform comparisons between healthy adults (a) and each immunosuppressed patient group (Bonferroni multiple comparisons test; a–b, a–c: $p < 0.001$, a–d: $p = 0.002$, a–e: $p = 0.009$).

Table 2. Kinetics of the neutralizing antibody titers.
Neutralizing antibody titers against wild-type and omicron proteins were measured using the plaque reduction neutralization test on the sera from healthy adults and immunocompromised hosts before and at 3 and 6 months after the third vaccination). The results are expressed as the median titer (interquartile range), percentage of positive and negative responses, and number of responders per group and per time point. A cutoff value of 1:8 was considered a positive response.

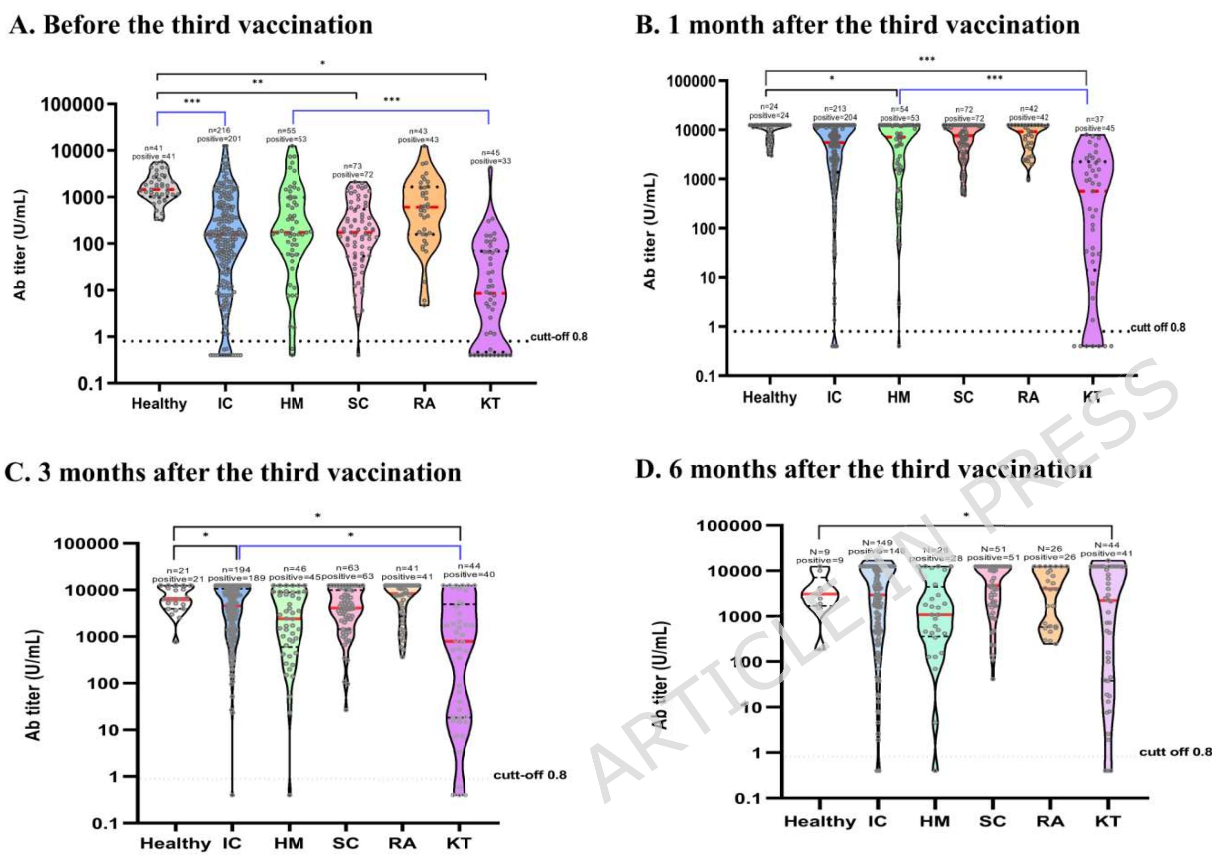
		Healthy[a] (n = 10)				Immunocompromised host (45)				p-value		Hematologic malignancy[b] (n = 13)				Solid cancer[c] (n = 10)				Rheumatoid arthritis[d] (n = 10)				Kidney transplantation[e] (n = 12)				p-value	
		Wild		Omicron		Wild		Omicron		Wild	Omicron	Wild		Omicron		Wild		Omicron		Wild		Omicron		Wild		Omicron		Wild	Omicron
		Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative			Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative		
Before the third vaccination	n/N(%)	5/6(83.33)	1/6(16.67)	5/6(83.33)	1/6(16.67)	33/45(73.33)	12/45(26.67)	11/45(24.44)	34/45(75.56)	>0.9999*	0.0089*	13/13(100.00)	0/13(0.00)	4/13(30.77)	9/13(69.23)	7/10(70.00)	3/10(30.00)	2/10(20.00)	8/10(80.00)	6/10(60.00)	4/10(40.00)	5/10(50.00)	5/10(50.00)	7/12(58.33)	5/12(41.67)	0/12(0.00)	12/12(100.00)	0.0696*	0.0502*
	Median (IQR)	1:128.0 (1:24.25–1:128)		1:8.0 (1:6.25–1:32)		1:16 (1:1–1:128)		1:1 (1:1–1:6.25)		0.127**	0.008**	1:16 (1:16–1:192)		1:1 (1:1–1:8)		1:32 (1:1–1:160)		1:1 (1:1–1:2.75)		1:32 (1:1–1:224)		1:4.5 (1:1–1:64)		1:8 (1:1–1:8)		1:1 (1:1–11)		0.017***	0.002***
1 month after	n/N(%)	10/10(100.00)	0/10(0.00)	10/10(100.00)	0/10(0.00)	43/48(89.58)	5/48(10.42)	32/45(71.11)	13/45(28.89)	0.5753*	0.0950*	13/13(100.00)	0/13(0.00)	10/13(76.92)	3/13()	10/10(100.00)	0/10(0.00)	7/10(70.00)	3/10(30.00)	10/10(100.00)	0/10(0.00)	10/10(100.00)	0/10(0.00)	10/12(83.33)	2/12(16.67)	5/13(38.46)	7/13(53.85)	0.1241*	0.1250*
	Median (IQR)	1:512 (1:256–1:1280)		1:128 (64–320)		1:256 (1:64–1:1024)		1:32 (1:1–1:160)		0.239**	0.018**	1:1024 (1:272–1:1536)		1:128 (1:4.5–1:256)		1:384 (1:224–`1:2048)		1:32 (1:1–1:256)		1:256 (1:128–1:2560)		1:80 (1:28–1:1280)		1:64 (1:10–1:256)		1:1 (1:1–1:12)		0.012***	0.001***
3 months	n/N(%)	10/10(100.00)	0/10(0.00)	9/10(90.00)	1/10(10.00)	43/45(95.56)	2/45(4.44)	28/45(62.22)	17/45(37.78)	>0.9999*	0.1399*	13/13(100.00)	0/13(0.00)	9/13(69.23)	4/13(30.77)	10/10(100.00)	0/10(0.00)	6/10(60.00)	4/10(40.00)	10/10(100.00)	0/10(0.00)	7/10(70.00)	3/10(30.00)	10/12(83.33)	2/12(16.67)	6/12(50.00)	6/12(50.00)	0.1241*	0.7261*

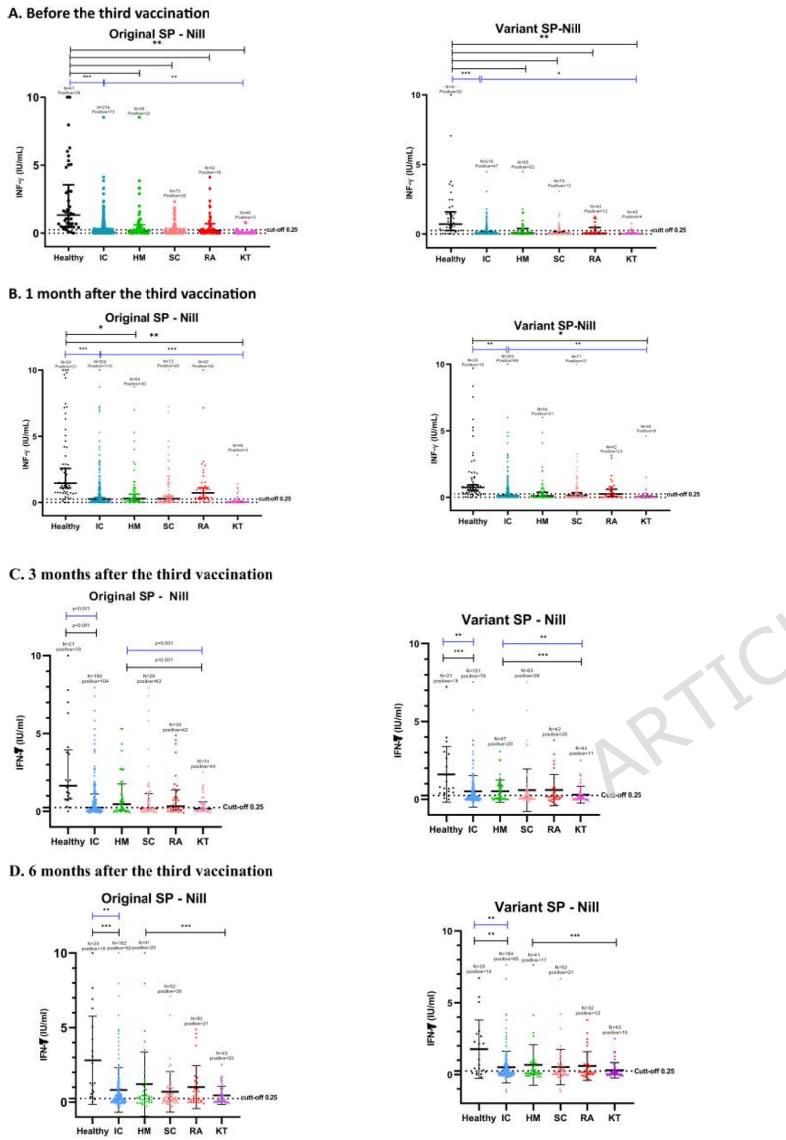
after	Median (IQR)	1:807 (1:256–1:1024)		1:64 (28–160)		1:157 (1:61–1:768)		1:16 (1:1–1:128)		0.054**	0.157**	1:218 (1:93–1:1024)		1:32 (1:1–1:192)		1:192 (1:32–1:512)		1:16 (1:1–1:32)		1:2213 (1:100–1:8192)		1:544 (1:1–1:1024)		1:64 (1:10–1:256)		1:5 (1:1–1:52)		0.021***	0.070***
6 months after	n/N(%)	9/9(100.00)	0/9(0.00)	9/9(100.00)	0/9(0.00)	37/42(88.10)	5/42(11.90)	30/42(71.43)	12/42(28.57)	0.5712	0.0940	9/10(90.00)	1/10(10.00)	8/10(80.00)	2/10(20.00)	9/10(90.00)	1/10(10.00)	7/10(70.00)	3/10(30.00)	10/10(100.00)	0/10(0.00)	7/10(70.00)	3/10(30.00)	9/12(75.00)	3/12(25.00)	8/12(66.67)	4/12(33.33)	0.3363*	0.9160*
	Median (IQR)	1:939 (1:369–1:8192)		1:64 (16–640)		1:256 (1:128–1:8192)		1:48 (1:1–1:512)		0.207	0.428	1:640 (1:253–1:8192)		1:48 (1:12–1:256)		1:256 (1:154–1:2560)		1:12 (1:1–1:352)		1:2176 (1:112–1:8192)		1:288 (1:1–1:640)		1:384 (1:3–1:7168)		1:96 (1:1–1:896)		0.596***	0.786***

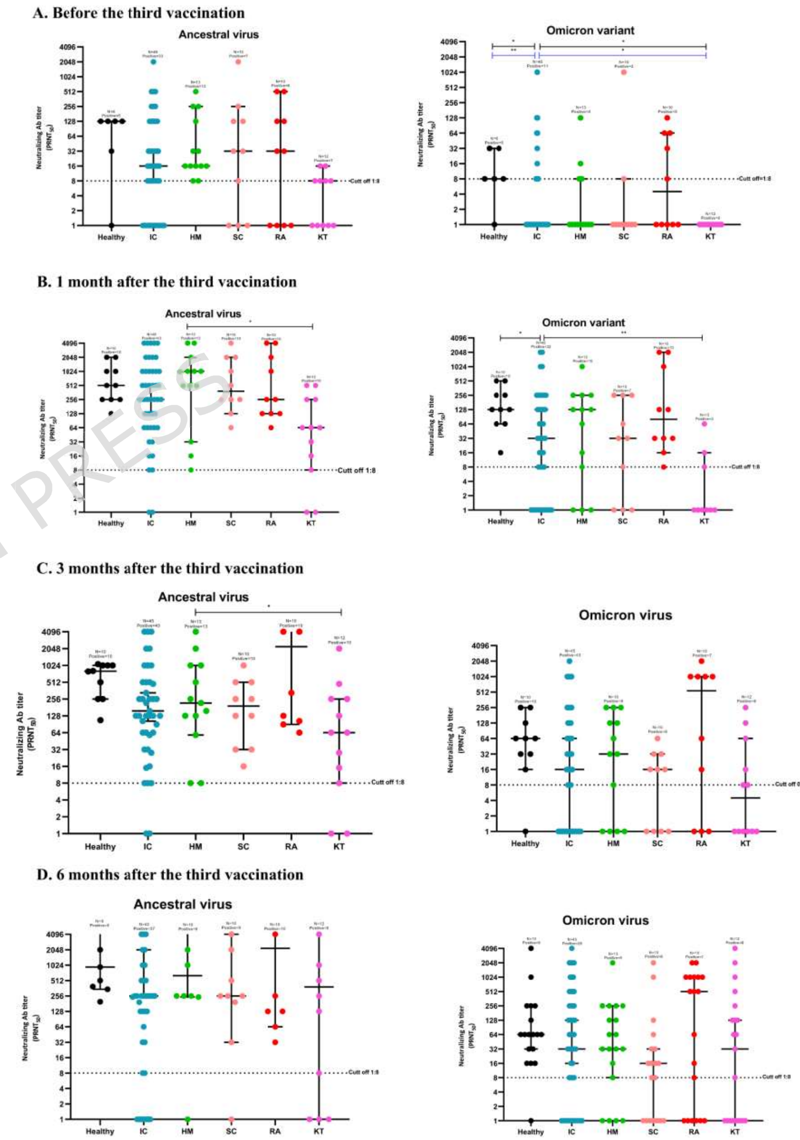
* Fisher's exact test was used to perform comparisons between the healthy and immunosuppressed groups and among the immunocompromised groups using categorical data (n/N (%)).

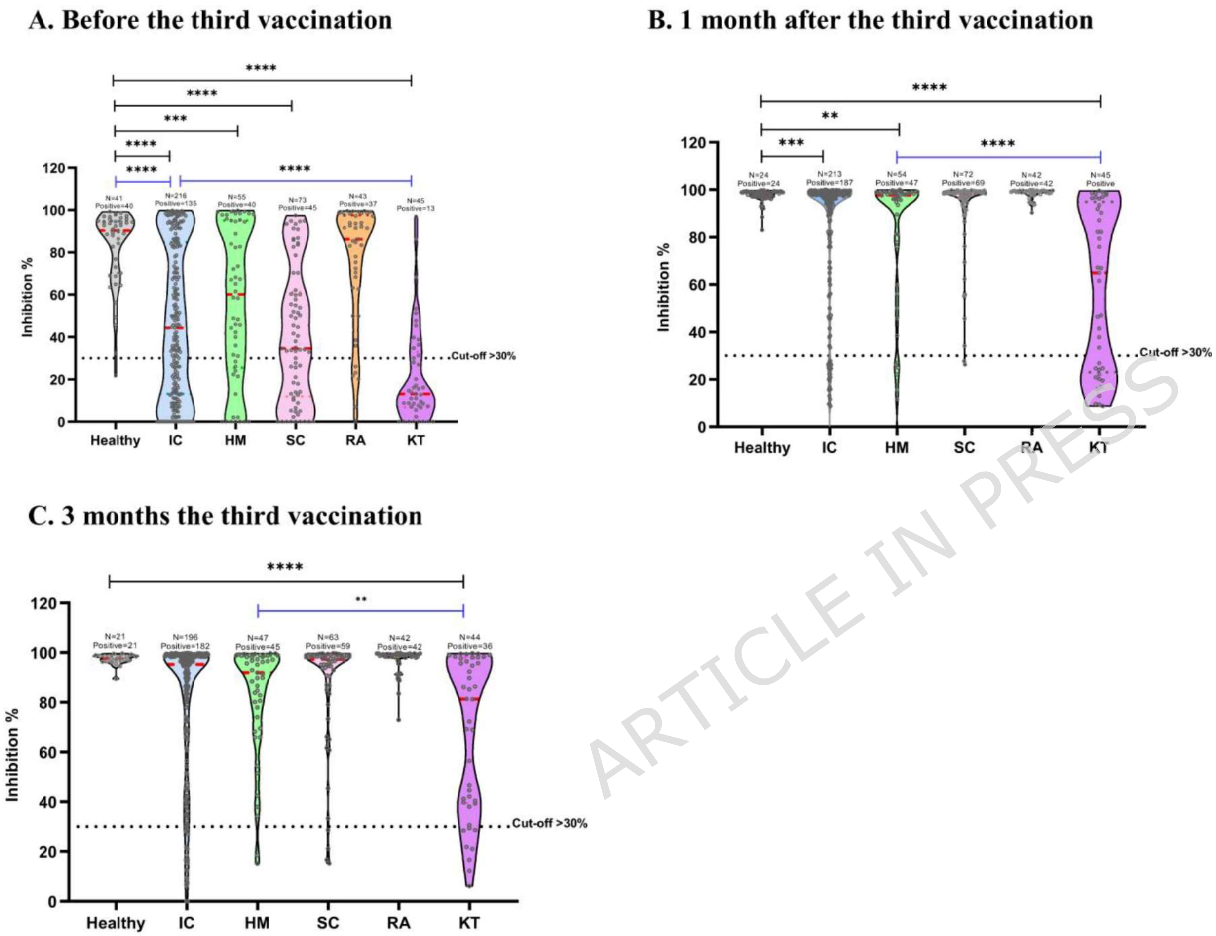
** The Mann–Whitney U test was used to perform comparisons between the healthy and immunosuppressed groups using numerical data (median, interquartile range (IQR)).

*** The Kruskal–Wallis test was used to perform comparisons between antibody titers for spike protein binding before and 1 month after the third vaccination in immunosuppressed patients and healthy adults (Bonferroni multiple comparisons test before the third vaccination: a–b, a–c, a–e: $p < 0.001$, a–d: $p = 0.010$; 1 month after: a–b: $p = 0.002$, a–c: $p = 0.021$, a–d: $p = 0.281$, a–e: $p < 0.001$; 3 months after: a–b: $p = 0.008$, a–c: $p = 0.122$, a–d: $p = 0.940$, a–e: $p < 0.001$; 6 months after: a–b: $p = 0.294$, a–c: $p = 0.425$, a–d: $p = 0.820$, a–e: $p = 0.550$) using numerical data (median, IQR).


A. Before the third vaccination
Ab titer (U/mL)
100000
10000
1000
100
10
1
0.1
n=41 positive =41
n=216 positive=201
n=55 positive=53
n=73 positive=72
n=43 positive=43
n=45 positive=33
cutt-off 0.8
Healthy
IC
HM
SC
RA
KT
B. 1 month after the third vaccination
Ab titer (U/mL)
n=24 positive=24
n=213 positive=204
n=54 positive=53
n=72 positive=72
n=42 positive=42
n=37 positive=45
cutt off 0.8
C. 3 months after the third vaccination
Ab titer (U/mL)
n=21 positive=21
n=194 positive=189
n=46 positive=45
n=63 positive=63
n=41 positive=41
n=44 positive=40
cutt-off 0.8
D. 6 months after the third vaccination
Ab titer (U/mL)
N=9 positive=9
N=149
N=51 positive=51
N=26 positive=26
N=44 positive=41
cutt off 0.8


A. Before the third vaccination
Original SP - Nill
Variant SP-Nill
INF-γ (IU/mL)
Healthy
IC
HM
SC
RA
KT
cut-off 0.25
B. 1 month after the third vaccination
Original SP - Nill
Variant SP-Nill
C. 3 months after the third vaccination
Original SP - Nill
Variant SP - Nill
IFN-γ (IU/ml)
Cutt-off 0.25
D. 6 months after the third vaccination
Original SP - Nill
Variant SP - Nill


A. Before the third vaccination
Ancestral virus
Omicron variant
Neutralizing Ab titer (PRNT50)
Healthy
IC
HM
SC
RA
KT
Cutt off 1:8
B. 1 month after the third vaccination
Ancestral virus
Omicron variant
C. 3 months after the third vaccination
Ancestral virus
Omicron virus
D. 6 months after the third vaccination
Ancestral virus
Omicron virus


A. Before the third vaccination
****
****
***
****
****
****
Inhibition %
N=41 Positive=40
N=216 Positive=135
N=55 Positive=40
N=73 Positive=45
N=43 Positive=37
N=45 Positive=13
Cut-off >30%
Healthy
IC
HM
SC
RA
KT
B. 1 month after the third vaccination
****
**
***
****
Inhibition %
N=24 Positive=24
N=213 Positive=187
N=54 Positive=47
N=72 Positive=69
N=42 Positive=42
N=45 Positive
Cut-off >30%
Healthy
IC
HM
SC
RA
KT
C. 3 months the third vaccination
****
**
Inhibition %
N=21 Positive=21
N=196 Positive=182
N=47 Positive=45
N=63 Positive=59
N=42 Positive=42
N=44 Positive=36
Cut-off >30%
Healthy
IC
HM
SC
RA
KT