의학박사 학위논문

Impact of Frailty on Immune Response to Varicella-Zoster Vaccine in Community-dwelling Older Adults

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대상포진백신 (조스타박스) 면역반응에 노쇠가 미치는 영향

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ABSTRACT

Introduction: The higher incidence rate of herpes zoster in older individuals correlates with a decline in varicella-zoster virus (VZV)-specific T-cell-mediated immunity. To evaluate whether, in older individuals, the immunogenicity of the herpes zoster live-attenuated vaccine (ZVL) is influenced by frailty status or baseline cytokine levels, individuals aged ≥65 years were prospectively enrolled.

Methods: After undergoing evaluation for frailty status (K-FRAIL scale), cytokine levels, and comprehensive geriatric assessment, the individuals enrolled for the study were administered with ZVL from April 2016 to September 2016. Humoral and cellular immunogenicity were analyzed using enzyme-linked immunosorbent assays (ELISAs) for the VZV-specific IgG antibody and VZV-specific enzyme-linked immunospot (ELISPOT) assays, respectively, six weeks before and after the administration of ZVL. Cytokine (transforming growth factor-alpha, interleukin-6, and interleukin-1ß) levels and high-sensitivity C-reactive protein (hsCRP) levels were measured using highly sensitive multiplex assay and turbidimetric immunoassay.

Results: Among 69 participants (mean age=74.3 years); 2, 29, and 38 participants were categorized as frail, pre-frail, and robust, respectively. Non-robust group (n=31) was defined as the composite of pre-frail and frail participants. Thirty-seven participants had a >2 geometric mean fold rise (GMFR) in IgG antibodies, 22 additional participants had ≥10 SFCs/10^6 PBMCs (Peripheral blood mononuclear cells), from 35/69 to 57/69 participants, and 42 experienced a two-fold GMFR in SFCs six-weeks after vaccination.

The GMFR in the antibody titers was similar in the robust and non-robust groups; moreover, the baseline ELISPOT assay geometric mean values were not significantly different before or after vaccination. We observed no significant correlations between baseline cytokine levels and immunogenicity.

Conclusion: Approximately half of the participants in this study experienced a two-fold rise in antibodies and additional one-third of the subjects showed ≥10 SFCs after ZVL administration, suggesting that the ZVL can boost immunity in non-robust older adults (especially pre-frail) as compared to robust older adults.

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Keywords: varicella-zoster vaccine, frailty, immunogenicity, immunosenescence, herpes zoster, cytokine

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국문 초록

서론: 노인 인구에서 대상포진의 높은 유병률은, 수두 바이러스에 대한 T-세포 면역 감소와 관련이 있다. 65 세 이상 노인 인구에서 노쇠 상태와 기저 혈중 사이토카인 농도에 따른 약독화 생수두백신 (조스타박스)의 면역 생성능을 확인 하고자 한다.

방법: 2016 년 4 월에서 2016 년 9 월까지, 분당서울대학교병원에서 65 세 이상 연구 참여 대상자를 전향적으로 모집하여 K-FRAIL (한국형 노쇠 설문지) 로 노쇠 상태를 평가 하고, 노인포괄평가, 혈중 사이토카인 농도를 측정 후 약독화 수두 생 백신을 접종 하였다. 노인포괄평가는 동반질환, 복용 약제, 악력, 보행속도, 일상생활 수행능력 (Activity of Daily Living, ADL), 도구적 일상생활 수행능력 (Instrumental Activity of Daily Living, IADL), 인지기능 평가, 노인 우울증 평가, 영양 평가, 섬망 발생 위험 평가 등으로 구성되어 있는 다학제적 평가 도구이다. 세포성,체액성 면역생성능은 효소결합면역점 (ELISPOT assay) 측정과 효소결합면역흡착층정법 (ELISA assay) 으로 백신 투여 전과,백신 투여 6 주후 확인 하였다.사이토카인은 고민감 복합 측정 (high-sensitive multiplex assay) 로 측정 하였고, 고민감 C-단백 반응 (hsCRP)은 비탁도 면역측정법 (turbidimetric immunoassays) 으로 측정하였다. 체액성면역은 대상포진바이러스에 특이적으로 결합하는 환자 혈액내 IgG 항체의역가와 (geometric mean value, GMV), 항체의증가배율(geometric mean fold rise, GMFR), 항체가 2 배이상증가한경우(2 ≥ GMFR) 를 기준으로 평가하였다. 세포성면역은 10^6 개의 말초 단핵세포 (PBMCs) 당 대상포진 항원에 반응하여 IFN-γ 를 생성하여 반응한 세포의 개수 (Spot Forming Cells, SFCs)로 확인 하였다. 우선 10 SFCs/10^6 PBMCs 의 세포 면역을 달성 하였는지 여부를 백신 투여 전/후에 확인 하였다. 이후에는 백신 투여 전 10 개 이상이었던 군과 10 개 이하였던 군으로 나누어 분석 하였다. 백신

투여 전 대상포진 항원에 반응하는 세포의 수가 10 SFCs/10^6 PBMCs 이하의 세포면역을 보인 환자들의 경우, 백신 투여 후 10 SFCs/10^6 PBMCs 이상으로 증가 하였는지를 확인 하였다. 백신 투여 전 대상포진 항원에 반응하는 세포의 수가 10 SFCs/10^6 PBMCs 이상인 경우, 백신 투여 전 후 10^6 PBMCs 당 반응하는 세포의 수 (geometric mean value, GMV), 반응 세포의 증가배율(geometric mean fold rise, GMFR), 반응세포가 2 배이상증가한경우(2 ≥ GMFR) 를 기준으로 평가하였다.

결과: 총 69 명의 (평균 연령 74.3 세)대상자가 연구에 참여 하였으며, 전체 환자 중 2 명, 29 명, 38 명의 참여자가 노쇠 (frail), 전노쇠 (pre-frail), 건강 (robust) 군으로 분리 되었다. 노쇠군과 전노쇠군의 합은 비-건강 (non-robust) 분으로 분류 되었으며 31 명의 환자가 이 군에 해당 하였다. 연구 결과 체액성 면역 지표로서 37 명의 참여자가 약독화 수두 생 백신 투여 6 주후 2 배 이상의 항체 생성능 (2 ≥ GMFR)을 나타내었으며, 세포성 면역 지표로서 10 SFCs/10^6 PBMCs 개 이상의 세포 면역을 보인 환자의 수가, 백신 투여 전에 35 명 비교하여 백신 투여 후 57 명으로, 22 명의 환자가 추가로 10 SFCs/10^6 PBMCs 개 이상의 세포 면역능을 가지게 되었다. 또한 42 명이 2 배 이상의 세포 면역능 증가가 (2 ≥ GMFR) 있었다. 항체의 증가비 (GMFR) 는 건강군과 비-건강군에서 비슷하였으며, 백신 투여 전과 6 주 후 세포면역수치 또한 통계적으로 유의한 차이를 보이지 않았다. 백신 투여 전 혈중 사이토카인 농도 상태와, 백신 투여 후 면역 생성능간에 상관관계는 관찰되지 않았다.

결론: 본 연구에서 지역사회 거주 고령자 참가자의 약 2/3 이상이 약독화 수두 생 백신 투여 후 세포 면역 생성을 보이고, 약 절반 이상이 항체가 2 배 이상을 달성 하였다. 또한 세포성 면역과 체액성 면역 생성 능력에서 비건강 노인군과 건강노인군에서 통계적으로 유의한 차이를 보이지 않았다. 이는

약독생수두백신이 지역사회 건강 그리고 비 건강 노인에서도 건강 노인과 비슷한 면역 증강을 유도 한다는 것을 시사 한다.

주요어: 수두 대상포진 바이러스, 노쇠, 면역노화, 면역생성능, 대상포진, 사이토카인

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CONTENTS

1. Introduction

Herpes zoster (HZ) is a common neuro-cutaneous disease with a worldwide incidence rate of 3-5/1000 person years. (1) Clinical presentation of HZ includes a vesicular rash accompanied by pain or burning sensation along the unilateral dermatome and chronic, long-lasting pain persisting for more than 120 days after the onset of rash, known as postherpetic neuralgia (PHN). PHN is a common complication of HZ. Patients who suffer from PHN may require prolonged analgesic use, and it can impair quality of life or daily functional capacity. The incidence and severity of HZ and PHN increase with age, as more than 60% of reported HZ cases occur in persons aged more than 50 years (2-4), and lifetime risk of HZ is as high as 50% in individuals over 85 years of age. (5-7) Age-associated increased risk of HZ correlates with a decline in Varicella Zoster Virus (VZV)-specific T-cell-mediated immunity, a phenomenon characteristic of immunosenescence. (8, 9)

It is recommended for older adults to receive live-attenuated VZV-containing vOka vaccine (ZVL, zoster vaccine live). The ZVL is licensed for use in population aged 50 and more and recommended for use in those ≥ 60 years of age by the Advisory Committee on Immunization Practices. The phase III Shingles Prevention Study (SPS) showed that the ZVL had an estimated vaccine efficacy (VE) of 51% (95% confidence interval [CI], 44%－58%) for reducing the incidence of HZ in persons aged ≥60 years. (10) Another phase III Zostavax Efficacy and Safety Trial (ZEST) was conducted to evaluate the VE of zoster vaccine in people aged 50－59 years and showed an estimated 70% VE (95% CI, 54%－81%). (11) The ZVL also led to decrease in other

complications including PHN (59%－67%). (10-13) However, only 26% of vaccinees aged over 80 years were protected from PHN indicating an age-related reduction in vaccine efficacy and that HZ vaccine is only partially protective in very old populations. Recently, a new herpes zoster vaccine (Zoster Vaccine Recombinant, Adjuvanted) with higher estimated efficacy (91.3%) as compared to ZVL (38%) in older adults aged over 70 years, was just approved (Oct 20, 2017), and is in the process of market release. (14)

Immunosenescence could be defined as age-related changes in innate and adaptive immune system, resulting in greater susceptibility to infection, reduced response to vaccination and it is known to lead to age-related inflammatory diseases, such as osteoporosis or atherosclerosis. (15) This age-related change in immune systems affects both the innate and adaptive immune systems, and manifests as alterations in both, the numbers and functions of the various immune cell types. (16) As a consequence of immunosenescence, influenza, pneumococcal pneumonia, and herpes zoster vaccines are less effective in the older adults as compared with young adults. (15) Previous research has showed that after influenza vaccination, the adjusted odds-ratios (OR) of seroconversion and seroprotection for all three antigens decreased (0.24 - 0.59) in older adults as compared to their younger counterparts. (17)

However, the old population is heterogeneous in terms of overall comorbidity, health status, functional capacity, and immunological response. Frailty manifests as vulnerability to possible stress, decreased homeostatic capacity and reflects physiological age rather than chronological age. (18, 19) Frailty is intimately associated with adverse clinical outcomes including increased mortality, functional dependence, or

institutionalization with increased public medical expenditure. Various methods and concepts to identify frailty have been introduced and validated, from single gait speed (20, 21) or frailty phenotype (19) to the frailty index (FI), which is calculated using more than 70 results obtained in a comprehensive geriatric assessment (CGA). (22) With respect to availability of an easy screening method for frailty status without additional physical examination or blood sampling, clinical validity and feasibility of Korean version of the FRAIL (K-FRAIL) scale, which is derived from Morley's FRAIL scale, has been reported. (23)

Theoretical models explaining the immunosenescence are immune risk profile (IRP), cellular senescence, and the senescence-associated secretory phenotype (SASP). (24, 25) Hallmarks of inflammation, including elevated IL-6, TNF-α, and immune cell chemokines, are associated with dementia (26), depression (27), atherosclerosis (28), cancer (29), diabetes (30), mortality (26, 31, 32), and frailty. (33) The processes of immunosenescence and frailty both contribute to morbidity and mortality in older adults through increased susceptibility to infections and reduced efficacy of vaccine. Frailty and immunosenescence are thought to share a common biological or pathological pathway including chronic inflammation, altered homeostasis, or suppressed diversity. Identifying the biomarkers of both immunosenescence and frailty should enable the construction of more effective vaccine formula and vaccination strategies for the older adults against infections. For example, potential biomarkers may help identify those who are likely to show a decreased efficacy to vaccination or are at risk of developing frailty. These

patients could potentially benefit from adjuvant strategies such as intradermal injection, higher doses or booster doses.

Thus, the primary purpose of this study was to evaluate the impact of frailty on ZVL-induced immunogenicity in community-dwelling older adults who visited the outpatient clinic of a tertiary hospital, using VZV-specific enzyme-linked immunosorbent assays (ELISAs) and VZV-specific interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISPOT) assay. The secondary objective of this study was to identify the relationship between biomarkers of frailty or immunosenescence and ZVL-induced immunogenicity.

2. Materials and Methods

Subjects and Vaccination

From April 2016 to September 2016, participants aged 65 years and older, who had given prior informed consent, were enrolled for the study. Exclusion criteria were: 1) known contraindication to live zoster vaccination, 2) previous zoster history in the past 10 years, 3) previous zoster vaccine, 4) history of taking immunosuppressive drugs, 5) HIV infection, 6) organ transplantation, 7) autoimmune disease, 8) individuals who had a significant underlying illness that would be expected to prevent completion of the study, 9) receipt of any other immunization within one month before the beginning of vaccination under this study or scheduled within 6 weeks after the beginning of vaccination under this study, 10) receipt of transfusion or antibodies within three months before the beginning of vaccination under this study or scheduled within 6 weeks after the beginning of vaccination under this study, and 11) other conditions that cause an abnormal immune response to vaccination. The live attenuated varicella-zoster vaccine (Zostavax® , MSD, Kenilworth, NJ, USA), ZVL, containing 19,400 plaque-forming units of the Oka/Merck strain, was injected subcutaneously once (0.65 mL). Blood samples were collected six weeks before and after vaccination into three heparin-containing vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ, USA) and one serum-separating vacutainer tube (SST, Becton Dickinson, Franklin Lakes, NJ, USA). On the day of blood collection, the blood was transferred to the laboratory in Seoul National University

Bundang Hospital. Peripheral blood mononuclear cells (PBMCs) were immediately separated on Ficoll-Hypaque (Uni-Sep, Novamed, Israel) medium at 1800 rpm for 20 minutes with break off. Serum was separated from SST by centrifuging the SST tubes at 300 rpm for 7 minutes and was divided in to 6 samples of 200 μL each. PBMCs and serum samples were immediately stored in liquid nitrogen at –70℃ with serum-free cryopreservation medium (Cellbanker 2, Zenoaq, Japan) as described previously. (34)

Immunologic Assessment

The IgG antibody titer against VZV glycoprotein was measured by using Serion Enzyme-linked Immunosorbent Assay (ELISA) Classic (Institute Virion\Serion, Würzburg, Germany) according to the manufacturer's instructions. Measurement range of ELISA kit was 15-2000 mIU/mL. Serum samples were diluted (1:1000) and added in duplicates into a 96-well plate coated with VZV-specific antigens and incubated at 37℃ for 1 h. After washing, anti-human IgG antibody conjugated to alkaline phosphatase was added to the wells and the plate was incubated at 37℃ for 30 min. After this, para-nitrophenyl phosphate solution was added, and the plate was incubated at 37℃ for 30 min. Optical density was measured at 405 nm using a VersaMax microplate spectrophotometer (Molecular Devices Corporation, CA, USA) and antibody was quantified using a lot-specific standard curve based on a 4-parameter logistic function. (Figure 1)

The VZV-specific IFN-γ enzyme-linked immunospot (ELISPOT) assay was performed using an IFN-γ ELISPOT set (BC Bioscience, San Jose, CA, USA), as described previously. (34) The 96-well plates were coated with 100 μL of 1:200 diluted anti-human IFN-γ monoclonal antibody (BD bioscience, San Jose, CA, USA) and then incubated overnight. After washing, the wells were blocked for 2 h with culture medium containing 10% fetal bovine serum and complete medium containing an ultraviolet-inactivated preparation of VZV antigen (VR-916, American Type Culture Collection, Manassas, VA, USA) derived from the clarified cell culture supernatants of VZV-infected MRC-5 cells. The negative control antigen was produced via the same process but using the uninfected MRC-5 cells. Phytohemagglutinin (PHA) was incorporated as a positive control.

The PBMCs (100 μL, 10^7/mL) of the participants were added in duplicates, and plates were incubated for 16-20 hours at 37℃ with 5% CO_2 and 95% humidity. The control sample of PBMCs from healthy young adults (30 years old, male without any disease) was analyzed together with the other samples for each experiment, to evaluate the appropriateness of the experiment. The plates were washed, and then 100 μL of biotinylated anti-human IFN-γ antibody was added to each well. Plates were incubated for 2 h at 4℃, after which they were washed. For color development, streptavidin-horseradish peroxidase enzyme and its substrate (AEC Substrate Reagent set, BD Bioscience, San Jose, CA, USA) were added and kept for 7-10 minutes, after which the reaction was stopped using dextrose water. The resulting spots were enumerated by using an automated microscope (CRL ImmunoSpot S4Core Analyzer, Cellular Technology ltd., Cleveland, OH, USA). The VZV-specific responses were reported as spot-forming cells

(SFCs) per 10^6 PBMCs (i.e., SFC count in response to VZV antigen minus SFC count in response to MRC-5 cell antigen) and samples with a response to PHA (<300 SFCs/10^6 PBMCs) were excluded from the analysis. (Figure 2) A photo of the ELISPOT assay is presented in Figure 3.

For the analysis, humoral immunity was presented in terms of the level of serum VZV-specific IgG titer (geometric mean value, GMV, IU/mL), fold increase in IgG titer (geometric mean fold rise, GMFR) and increment of more than 2 fold in antibody titer (2 > GMFR). Cellular immunity was evaluated in terms of spot-forming cells (SFCs) producing IFN-γ in response to VZV-specific antigen per 10^6 peripheral blood mononuclear cells. First, sufficiency of cellular immunity was determined with cut-off of 10 SFCs/10^6 PBMCs, before and after vaccination. Then a separate group was formed including individuals with 10 SFCs/10^6 PBMCs for cellular immunity before vaccination. In the group of participants whose cellular immune response was less than 10 SFCs/10^6 PBMCs, it was confirmed whether the cellular immune response level after vaccination increased to 10 SFCs/10^6 PBMCs or more. In the other group (cellular immunity ≥10 SFCs/10^6 PBMCs before vaccination), cellular immune response or immunogenicity was presented in terms of reactive SFCs per 10^6 PBMCs (GMV), the increment in reactive SFCs (GMFR) and the increase in number of reactive cells by 2 fold or more (2 ≥ GMFR).

Quantification of Plasma Cytokines

A V-PLEX customized panel kit (Meso Scale Discovery [MSD], Rockville, Maryland, USA) was used according to the manufacturer's protocol for analysis of the stored serum samples. The concentration of three cytokines viz., transforming growth factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) was measured in duplicates and data were acquired using a SECTOR S 600 plate reader (Meso Scale Discovery, Rockville, Maryland, USA). High-sensitivity C-reactive protein (hsCRP) was measured by turbidimetric immunoassays. (Figure 4)

Comprehensive Geriatric Assessment (CGA) and Frailty Assessment

Before immunization, CGA encompassing six domains was carried out in geriatric department of Seoul National University Bundang Hospital. CGA is defined as a multidimensional, interdisciplinary, diagnostic, and therapeutic process focusing on medical, psychological, functional, and social capability to develop a coordinated and integrated plan for treatment and long term follow-up. Our CGA protocol included six domains: burden of comorbidity, medication, physical function, psychological status, nutrition, and risk of delirium as previously described. (35)

Comorbidity was assessed by the Charlson comorbidity index, a weighted index that considers the number and seriousness of comorbid diseases based on the risk of 1-year mortality. (36)

Physical function was evaluated by activities of daily living (ADLs), instrumental

ADLs (IADLs), gait speed, and grip strength. The ADLs were assessed using the modified Barthel Index, which was composed of 10 categories (grooming, bathing, eating, dressing, toilet use, fecal continence, urinary continence, ability to go upstairs, ability to go down stairs, and walking in a hallway). This index has a scale from 0 to 100, where 100 indicates full independence, 75 to 99 indicate partial dependence, and less than 75 indicates full dependence. (37) The IADLs were measured using the Lawton and Brody Index, which includes five categories for men and eight categories for women (using the telephone, shopping, traveling via car or public transportation, using medication, and managing finances. Preparing food, doing laundry, and housekeeping categories were only for the women). Individuals who have at least 1 IADL scored as dependent were assumed to have IADL dependence. (38)

The gait speed was calculated from the time taken to walk 4.5 m of space after walking through one meter of unmeasured acceleration space. To minimize any potential bias, gait speed was measured by using an automated laser-gated chronometer attached to the wall. Handgrip strength (kg) was measured using a Jamar Plus+ Digital Hand Dynamometer (Sammons Preston, Bolingbrook, IL, USA) and the larger value of the two measurements taken for the dominant hand was used for further analysis. We adopted cut-off values for handgrip strength (<28.6 kg and 16.4 kg in male and female, respectively) from previous research based on data from the Korea National Health and Nutrition Examination Survey Ⅵ to identify decreased grip strength for sarcopenia. (39)

Psychological status was evaluated by the Korean version of the Mini-Mental State Examination, with a score ranging from 0 to 30 (a score less than 17 indicates dementia

and a score from 17 to 24 indicates mild cognitive impairment). (40) Depression was assessed by the short form of the Korean version of Geriatric Depression Scale; with scores ranging from 0 to 15 (a score of 9 or greater indicates severe depression and a score of 5 to 8 indicates mild depression). (41)

Nutritional status was analyzed using the Mini Nutritional Assessment (MNA), a validated nutrition screening and assessment tool that can identify geriatric patients who are malnourished or at risk of malnutrition. MNA has a score ranging from 0 to 30 (a score less than 17 indicates malnutrition and scores of 17 to 23.5 suggests a risk of malnutrition). (42)

Risk of post-operative delirium was estimated by the Nursing Delirium Screening Scale; with scores ranging from 0 to 5 (a score more than 2 indicates an increased risk of postoperative delirium). (43)

In addition, a comprehensive medical history, the number of medications being taken, the patients' living situation, and information regarding social support was obtained during an interveiw of participants or their respective caregivers. A list of medications was provided by the patients or caregivers, and pharmacists compiled a list of potentially inappropriate medications for the older adults. Polypharmacy is defined as regular use of more than five medications by a patient.

We used a validated self-report frailty questionnaire, based on the Korean version of the FRAIL scale (K-FRAIL, an acronym for fatigue, resistance, ambulation, illnesses, and loss of weight). The scale assigns scores from 0 to 5 and classifies participants as robust

(score of 0), pre-frail (score of 1-2), and frail (score of 3 and more). (23, 44) Non-robust group was defined as a composite group of frail and pre-frail participants. Details of categories evaluated in the CGA have been summarized in Table 1.

Safety Assessment

Safety of the vaccine was evaluated based on type and severity of local and systemic adverse events with the help of a self-report structured questionnaire and a medical interview conducted six weeks after vaccination. Adverse events were categorized using a standard toxicity scale of the Food and Drug Administration. (45) The correlation between adverse events and vaccination was divided into three categories: unlikely, possible, or likely relationship. A vaccine-related adverse event was defined as having a possible or likely relationship.

Statistical Analysis

The independent t-test or Mann-Whitney U test was used to compare the continuous variables and Chi-square test or Fisher's exact test was used for comparing the categorical variables, as appropriate. Associations between continuous variables were determined using Pearson’s correlation analysis. The VZV-specific IgG levels and SFCs of the ELISPOT assay were represented as the geographic mean value (GMV) and

geometric mean ratio of values at baseline and six weeks after vaccination (the geometric mean fold-rise [GMFR]). Continuous variables are expressed as mean (standard deviation [SD]or 95% confidence interval [95% CI]) or median (interquartile range [IQR]) for variables not normally distributed. According to K-FRAIL scale, participants were grouped as robust versus non-robust (pre-frail to frail). All tests were two-sided, and $p<0.05$ was considered significant. The statistical analyses was performed using SPSS version 21.0 (IBM, Armonk, NY, USA). This study protocol was reviewed and approved by the institutional review board of Seoul National University Bundang Hospital (IRB No. B-1509/313-006) and registered at ClinicalTrials.gov (number: NCT02704572).

3. Results

Clinical Characteristics of Subjects

Informed consent was obtained from 78 potential subjects and they were enrolled in the study. Before vaccination, eight participants withdrew their consent and one person was excluded after a past ZVL administration was revealed; hence, data from 69 participants were used in the final analysis (Figure 5). The clinical characteristics of 69 subjects are shown in Table 2. The median age (IQR) was 73 years (69 - 79) with 32 (46.4%) subjects being≥ 75 years old. Thirty-five subjects (50.7%) were female.

Comprehensive Geriatric Assessment and Frailty Assessment

Among the 69 participants, 38 (55.1%) were determined as robust, 29 (42.0%) as pre-frail, and 2 (2.9%) as frail according to the K-FRAIL scale. The numbers of patients based on a K-FRAIL score was 38 with a score of 0 (55.1%), 21 with a score of 1 (30.4%), 8 with score of 2 (11.6%), and 2 with score of 3 (2.9%). The prevalence rates for positivity of each category of the K-FRAIL scale were 14 for fatigue (20.3%), 14 for resistance (20.3%), 12 for ambulation (17.4%), 1 for illness (1.4%), and 2 for loss of weight (2.9%). Baseline demographic and functional, physical, and laboratory characteristics grouped by frailty status using the K-FRAIL scale are represented in Table

3. Participants in non-frail group and pre-frail to frail group were not significantly different with respect to age, underlying diseases, body mass index (BMI), mid arm circumference (MAC), calf circumference (CC), nutritional state, and status of polypharmacy. Subjects of pre-frail to frail group were more likely to be of female gender and have lower grip strength, slower gait speed, lower cognitive function screening score (K-MMSE), and higher depressive screening score (SGDS).

Immunogenicity of Herpes Zoster Vaccine according to Frailty Status

Humoral and cellular immunogenicity of ZVL were measured on the basis of IgG antibody titers against VZV glycoprotein measured by ELISA and VZV-specific IFN-γ enzyme-linked immunospot (ELISPOT) assay six weeks before and after vaccination.

Among the participants, the mean GMV (95% confidence interval [CI]) of VZV-specific IgG antibody titer was 112.67 (81.70-143.65) IU/mL at baseline (before vaccination) and 244.50 (186.58-302.42) IU/mL at six weeks after vaccination. Thirty-seven (53.6%) participants showed at least two fold rise (GMFR ≥ 2) in antibody titer.

The mean GMFR in the titer was 3.09 (2.47-3.71). In both robust and non-robust groups, half of the zoster vaccine recipients had at least an increase in antibody titer of VZV by two-fold. (Figure 6)

Humoral immunogenicity of herpes zoster vaccine was compared between robust participants and non-robust participants according to the K-FRAIL scale. (Table 4) There were no significant differences between the robust and non-robust groups with respect to median GMV (baseline 79.62 IU/mL vs. 69.16 IU/mL, p=0.398 and six weeks after vaccination; 166.81 IU/mL vs.155.61 IU/mL, p=0.736) and median GMFR (2.00 vs. 2.30, p=0.210).

For the cellular immunogenicity measured by VZV-specific IFN- γ ELISPOT analyses before and six weeks after vaccination, the mean GMVs were 25.93 (95% CI: 17.86 – 34.00) SFCs/10^6 PBMCs and 45.74 (95%: 34.31–57.16) SFCs/10^6 PBMCs, respectively. The mean GMFR after vaccination was 6.19 (95% CI; 3.90–8.48). Thirty-five (50.7%) and fifty-seven (82.6%) participants had ≥10 SFCs/10^6 PBMCs at baseline and six weeks after ZVL vaccination, respectively; 42 subjects (60.9%) experienced a two-fold increase of GMFR according to the ELISPOT assay

The median GMVs according to the ELISPOT assay were 8.3 (IQR: 2.9–53.1) and 12.5 (IQR: 3.5–26.0) at baseline (p=0.995 by Mann-Whitney test) and 26.3 (IQR: 14.5–67.5) and 28.0 (IQR: 11.5–66.5) six weeks after vaccination (p=0.542 by Mann-Whitney test) in the robust and non-robust groups, respectively. There were no significant differences between the robust and non-robust groups in terms of median GMFR (2.92 vs. 2.44, p=0.530) and the number of participants with a GMFR >2 (65.8% vs. 54.8%, p=0.354, Figure 7).

Among 20 robust and 14 non-robust group participants with <10 SFCs per 10^6 PBMCs at baseline, five (25.0%) and four (28.6%) participants, respectively, still had <10 SFCs per 10^6 PBMCs six weeks after vaccination (p=1.000, by Fisher's exact test). Post-vaccination data for the 35 participants with ≥10 SFCs per 10^6 PBMCs at baseline according to frailty status are shown in Table 5. Immunologic response to ZVL according to the three frailty groups (robust, pre-frail and frail) is recorded in Table 6.

Impact of Frailty on Immunogenicity of Herpes Zoster Vaccine and Baseline Cytokine Level according to Age Groups

Additionally, subgroup analysis for groups 65≤age<80 and age≥80 was conducted to identify whether frailty status in younger subgroups has the same immunological burden as compared to frailty status in the older subgroups.

In the group of 65≤age<80 (n=53), the mean GMV of VZV-specific IgG antibody titer was 99.05 (67.22-130.88) IU/mL and 122.16 (48.01-196.30) IU/mL at baseline (before vaccination) and 213.78 (136.5-291.0) IU/mL and 264.93 (156.50-373.37) IU/mL at six weeks after vaccination in the robust (n=30) and non-robust (n=23) groups, respectively. The mean GMFR in the titer was 2.91 (2.00-3.82) and 3.70 (2.39-5.02). As per the ELISPOT assay, the mean SFCs/10^6 PBMCs were 30.28 (15.78-44.78) SFCs/10^6 PBMCs and 23.39 (9.76-37.0) SFCs/10^6 PBMCs at baseline (before vaccination) and 50.10 (29.20-71.00) SFCs/10^6 PBMCs and 35.87 (22.26-49.48) SFCs/10^6 PBMCs at six weeks

after vaccination in the robust and non-robust groups, respectively. The mean GMFR in the SFCs/10^6 PBMCs was 7.44 (2.98-11.89) and 5.56 (1.76-9.36).

In the group of age≥80 (n=16), the mean GMV of VZV-specific IgG antibody titer was 181.77 (39.33-324.22) IU/mL and 67.40 (40.50-94.30) IU/mL at baseline (before vaccination) and 366.05 (40.05-692.05) IU/mL and 179.42 (93.14-265.70) IU/mL at six weeks after vaccination in the robust (n=8) and non-robust (n=8) groups, respectively. The mean GMFR in the titer was 1.97 (1.40-2.54) and 3.12 (0.80-5.44). As per the ELISPOT assay, the mean SFCs/10^6 PBMCs were 27.31 (0-56.76) SFCs/10^6 PBMCs and 15.5 (5.96-25.05) SFCs/10^6 PBMCs at baseline (before vaccination) and 47.69 (15.52-79.86) SFCs/10^6 PBMCs and 55.81 (3.79-107.84) SFCs/10^6 PBMCs at six weeks after vaccination in the robust and non-robust groups, respectively. The mean GMFR in the SFCs/10^6 PBMCs was 5.49 (1.50-9.48) and 3.40 (0.98-7.02).

However, there was no statistically significant difference between robust and non-robust groups in both younger (65≤age<80) and older (age≥80) subgroups as determined by Mann-Whitney test. (Tables 7 and 8)

Impact of Comorbidity and Nutritional Status on Immunogenicity of Herpes Zoster Vaccine

Immunogenicity of ZVL according to comprehensive geriatric assessment included additional analyses of comorbidity status (CCI) and nutritional status (mid-arm circumference). Serum IL-6, TNF-α, and hsCRP were elevated in the older adults with a

chronic disease, which indicated trends similar to those in a previous study. (46)

For the comorbidity status (CCI=0 vs. CCI≥1), the mean GMV of VZV-specific IgG antibody titer were 111.89 (67.27-156.51) IU/mL and 113.76 (69.98-157.54) IU/mL at baseline (before vaccination) and 204.76 (143.44-266.09) IU/mL and 299.31 (188.67-409.95) IU/mL at six weeks after vaccination in the group of CCI=0 (n=40) and CCI≥1 (n=29) group, respectively. The mean GMFR in the titer was 3.09 (2.21-3.98) and 3.09 (2.20-3.97). According to ELISPOT assay, the mean SFCs/10^6 PBMCs were 28.98 (16.50-41.47) SFCs/10^6 PBMCs and 21.72 (12.52-30.93) SFCs/10^6 PBMCs at baseline (before vaccination) and 45.39 (29.11-61.67) SFCs/10^6 PBMCs and 46.22 (29.72-62.73) SFCs/10^6 PBMCs at six weeks after vaccination in the group of CCI=0 (n=40) and CCI≥1, respectively. The mean GMFR in the SFCs/10^6 PBMCs were 5.57 (3.10-8.06) and 7.03 (2.61-11.46).

In the category of the length of mid-arm circumference (MAC >27 vs. MAC≤27), the mean GMV of VZV-specific IgG antibody titer was 107.76 (40.97-174.56) IU/mL and 115.29 (81.59-149.00) IU/mL at baseline (before vaccination) and 235.37 (157.74-313.00) IU/mL and 249.37 (168.70-330.05) IU/mL at six weeks after vaccination in the group of MAC >27(n=24) and MAC≤27 (n=45) group, respectively. The mean GMFR in the titer was 3.65 (2.42-4.87) and 2.80 (2.08-3.51). According to the ELISPOT assay, the mean SFCs/10^6 PBMCs were 23.21 (8.82-37.60) SFCs/10^6 PBMCs and 27.38 (17.27-37.48) SFCs/10^6 PBMCs at baseline (before vaccination) and 35.56 (17.86-53.27) SFCs/10^6 PBMCs and 51.17 (36.17-66.16) SFCs/10^6 PBMCs at six weeks after vaccination in the

groups of MAC >27 and MAC≤27, respectively. The mean GMFR in the SFCs/10^6 PBMCs were 7.92 (2.56-13.29) and 5.26 (3.07-7.45).

There was no statistically significant difference in immunity to HZ, immunogenicity of ZVL or serum cytokine levels between the groups of CCI=0 vs. CCI ≥1 or MAC >27 vs. MAC≤27 by Mann-Whitney test. (Tables 9 and 10)

Immunogenicity of Herpes Zoster Vaccine and Baseline Cytokine Level according to Age Groups

Cellular and humoral ZVL immunogenicity were compared between participants aged <80 and ≥80 years based on the reference of a previous study that immunogenicity is reduced at age 80 and above (15). Median age was 72 (IQR 68.5–75.5, n=53) and 81.5 (IQR 80.25–84.75, n=16) in groups 65≤age<80 and age≥85, respectively.

The mean GMV of VZV-specific IgG antibody titer was 109.08 (73.54-144.62) IU/mL and 124.59 (54.05-195.12) IU/mL at baseline (before vaccination) and 235.98 (173.66-298.30) IU/mL and 272.73 (117.18-428.29) IU/mL at six weeks after vaccination in the group of 65≤age<80 and age≥85, respectively. The mean GMFR in the titer was 3.26 (2.51-4.00) and 2.55 (1.46-3.63). The mean ELISPOT SFCs/10^6 PBMCs were 27.29 (17.46-37.13) SFCs/10^6 and 21.41 (7.54-35.27) SFCs/10^6 at baseline (before vaccination) and 43.83 (30.94-56.91) SFCs/10^6 and 51.75 (25.02-78.48) SFCs/10^6 at six weeks after

vaccination in the group of 65≤age<80 and age≥85, respectively. The mean GMFR in the SFCs/10^6 was 6.62 (3.70-9.55) and 4.75 (2.53-6.96).

There was no difference in the rate of increase in SFC from <10 to ≥10 between the age groups post-ZVL. Furthermore, no significant differences were noted in ZVL humoral or cellular immunogenicity and baseline cytokine level according to the age groups, as measured via GMV and GMFR changes by Mann-Whitney test. (Table 11)

Baseline Cytokine Levels in the Older Adults

Baseline cytokine levels (TNF-α, IL-6, and IL-1β) and hsCRP levels in all patients were analyzed. Among the participants, IL-1β levels of 23 participants were below fit curve range. However, the IL-6 and TNF-α levels were not below the fit curve range. Distribution of the cytokine levels in all the participants are presented in Figure 8.

Mean values of the cytokine and CRP levels in all participants were 0.052 pg/mL (n=46, 95% CI: 0.037-0.068), 0.679 pg/mL (n=69, 95% CI: 0.486-0.863), 2.905 pg/mL (n=69, 95% CI: 2.684-3.127), 0.120 mg/dL (n=69, 95% CI: 0.072-0.168)for IL-1β, TNF-α, IL-6, and hsCRP, respectively; IL-1β levels in 23 participants were undetectable. Cytokine levels were similar between the groups of participants aged <80 and ≥80 years (Table 11). Additionally, the mean IL-6, TNF-α, and hsCRP levels were lower in the robust group than in the non-robust group, but without statistical significance (Table 12, Figure 9).

Immunogenicity of Herpes Zoster Vaccine according to the Baseline Cytokine Level

Humoral and cellular immunogenicity of ZVL were defined as the GMFR of the VZV-specific IgG antibody titer and the difference in SFCs (ΔSFCs/10^6 PBMCs) based on ELISPOT assay, before versus after six weeks of vaccination. Compared to the baseline results, the Pearson correlation coefficients for IL-6, TNF-α, and hsCRP were -0.027, -0.096, and -0.149, respectively, for the IgG antibody titer GMFR (p=0.823, p=0.433, and p=0.221, respectively) and -0.029, 0.022, and -0.011, respectively, for ΔSFCs (p=0.811, p=0.859, and p=0.927). (Figures 10 and 11).

Safety Assessment

There was one patient with a vaccine-related adverse event during the study period: the patient complained of urticaria after two weeks of vaccine injection (Toxicity Grade: Grade 1, Correlation between adverse events and vaccination: unlikely) There were no reports of zoster-like rashes during the study period.

4. Discussion

In our study, 37 participants (53.6%) showed more than two-fold increase in antibody titer in GMFR post-ZVL, and participants with ≥10 SFCs/10^6 PBMCs increased by 31.9%, from 35 participants (50.7%) to 57 participants (82.6%). Forty-two subjects (60.9%) experienced more than two-fold increase of GMFR in ELISPOT assay. While the present study is the first to evaluate cellular immunogenicity of ZVL in Korean individuals, our findings regarding humoral immunogenicity (GMFR of 3.1 [CI: 2.5–3.7]) were comparable to those of a previous population-based study in Korea (GMFR of 2.8 [CI: 2.5–3.1]). (47) Due to the lack of an established surrogate cut-off value for ZVL immunogenicity, we used previously published values (>10/10^6 PBMCs, GMV, GMFR, responders with ≥2-fold increase). (8)

While the Advisory Committee on Immunization Practices set no upper age limit for ZVL eligibility, some providers may be less likely to recommend the vaccine to persons who are very old or frail. This is due to the controversy regarding the immunogenicity or protective efficacy of ZVL, even though individuals who are very old or frail are at risk for HZ. For this reason, recently, a lot of research has been conducted regarding the use of an immune modulator with vaccination or a high dose vaccine to overcome immunosenescence in the older adults. (48-50) Identification of surrogate markers. which are associated with vaccine immunogenicity in older adults, will allow us to discriminate those at risk of vaccine failure and target those who require interventions such as immune modulation or high dose vaccination, accordingly.

Most of the knowledge on the decisive factors of vaccine efficacy or immunogenicity such as malnutrition (51, 52), disease status (52, 53), and frailty (54) in older adults comes from study of influenza vaccination. We have shown the specific evaluation of the impact of frailty and baseline pro-inflammatory cytokine levels on the ZVL immunogenicity in community-dwelling older adults. Although this is the first study to evaluate cellular immunogenicity of live attenuated herpes zoster vaccine in Korean, humoral immunogenicity of this study (mean GMFR of 3.1, 95% CI 2.5 – 3.7) was comparable from a population-based study in Korean (mean GMFR of 2.8, 95% CI 2.5-3.1). (46)

In this study, first, we found that the ZVL immunogenicity for participants over 80 years of age was comparable to those aged 65–80 years, at least among the community-dwelling ambulatory population. This is significant because older age groups are an important target population for anti-HZ vaccinations. This result suggests that if the older adults are living independently and available for ambulation, it is not necessary to hesitate against ZVL vaccination for aging reasons.

A major finding of this study was that, after ZVL administration, the immunogenicity boost in non-robust individuals was comparable to that in robust individuals, as assessed by the K-FRAIL score. There was no statistically significant difference in absolute baseline levels of cellular and humoral immunities, and immunogenicity after ZVL administration between these groups. While the proportion of frail participants was too small to derive a definitive conclusion, the immunogenicity of ZVL in the larger pre-frail group was comparable to that of the robust group in community-dwelling ambulatory

older individuals capable of visiting the outpatient clinic. This tendency was maintained in subgroup analysis based on age groups in both younger (65≤age<80) and older (age≥80) subgroups.

According to comorbidity burden and nutritional status, serum proinflammatory cytokine markers (IL-6, TNF-α, and hsCRP) were elevated in the older adults with a chronic disease. There was no significant difference in other baseline immunologic status and immunogenicity measures after vaccination according to chronic disease or nutrition status. These results provide a message that simply the chronic comorbidity status or nutritional assessment alone may be incomplete to assess future immunogenicity of ZVL.

Although Franceschi et al. (55) have proposed that immunosenescence is associated with an alteration in the cytokine milieu, which could be referred to as “inflamm-aging”, that favors a pro-inflammatory state and immunosenescence is known to be related with vaccine failure, our finding that baseline serum TNF-α, IL-6, and hsCRP levels were not associated with vaccine immunogenicity is consistent with the findings of a previous study conducted with ZVL. (56)

In this study, we also evaluated the relationships between cytokine levels and frailty in older Koreans for the first time to identify frailty biomarkers. We found that non-robust individuals tended to have higher IL-6, TNF-α, and hsCRP levels than individuals who are robust (Figure 9); these results were consistent with previous research (57).

Our study had several limitations. First, we could not establish with certainty whether vaccine-induced immunogenicity would confer protection from HZ onset or attenuate its severity. We mainly used GMV or GMFR for interpreting the immunologic results in this study instead of cut-offs that reflect clinical endpoint because even the largest study on ZVL conducted so far was unable to recruit the number of HZ cases required to accurately determine the immunologic cut-off value. (10, 11, 58) We additionally employed an arbitrary cut-off value of 10 SFC/10^6 PBMCs, which is a generally accepted value to interpret ELISPOT results of tuberculosis patients. Thus, vaccine efficacy, the ultimate outcome of ZEST or SPS study, could not be calculated because it requires estimated incidence from herpes zoster prevalence. Since this study led to identification of surrogate markers (cellular or humoral immunity), the result using cutoff values should be interpreted carefully. Therefore, it cannot be concluded that the vaccine efficacy in non-robust community-dwelling older adults is similar to that in the robust community-dwelling older adults. Second, although frail older individuals were sought for recruitment, our study only included 29 pre-frail (42%) and 2 frail participants (2.9%); these proportions were significantly lower than those in a previous study conducted in Korea (58.3% pre-frail and 17.6% frail participants). (22) Because the proportion of frail individuals was very small, separate statistical analysis of the immunogenicity of frail individuals was not feasible; hence, data of frail and pre-frail participants were pooled to form a non-robust group. The lack of statistical significance in our study between these groups might be attributable to the uneven recruitment of frail individuals. Participants in this study may not appropriately represent the entire community-dwelling older adults because they were robust enough to visit the tertiary hospital outpatient clinic and write

the consent form themselves. Thus, it cannot be assumed from results of this study that a frail older adult living in a nursing home or dwelling only at home can have similar immunogenicity to their pre-frail or robust counterparts. Third, due to the limited measurement range of the ELISA kit used, some serum samples (particularly four serum samples that were drawn before vaccination) may have been measured inaccurately. Fourth, even though AEs were evaluated six weeks following vaccination, the participants were given an emergency number to call whenever AEs occurred. Only one case of AE was reported. The lower incidence of AEs reported in the current study, compared to that in a previous study conducted in Korea, is most likely because of its mild severity and recall bias of participants. (47) Lastly, only the VZV-specific IFN-γ ELISPOT was used to evaluate VZV-associated cell-mediated immunity. Other methods, such as an intracellular cytokine assay, may reveal more details about the CD4/CD8 cell response and effector/memory cells. Thus, further prospective studies including large population with longer observation period, incidence of herpes zoster, and detailed investigation for immunologic markers are warranted.

Our study also has certain strengths. Data on cellular and humoral immunogenicity of ZVL among populations of Asian as well as Korean ethnicities are rare, especially in the older adults. To our knowledge, it is the first consecutively enrolled study to evaluate both cellular and humoral immune responses to ZVL and serum cytokine levels in older Koreans, with particular reference to frailty, age, comorbidity, and nutritional status. Although our study may not be generalized over race or ethnicity, our findings may

provide further insights in regard to immunogenicity of ZVL in non-robust (especially pre-frail) older adults.

5. Conclusion

Our data suggest that the ZVL boosts antiviral immunity in (at least) pre-frail community-dwelling older adults. Similarly, the ZVL immunogenicity in individuals aged over 80 years was comparable to that in individuals aged 65–80 years. Hence, ZVL shows comparable immunogenicity in non-robust (especially pre-frail) and older community-dwelling ambulatory individuals over 80 years of age.

Tables

Table 1. Component of Comprehensive Geriatric Assessment (CGA)

Component	Description
Medical	Problem list
	Charlson's Comorbidity Index
	Medication review
	Potentially Inappropriate Medication
Function	Basic activities of daily living (ADL)
	Instrumental activities of daily living (IADL)
	Gait speed
	Grip strength
Psychological status	Cognitive function (Mini-mental status examination, MMSE-KC)
	Depression (Korea Geriatric Depression Scale, SGDS-K)
	Nursing Delirium Screening Scale (NuDESC)
Nutrition	Mini nutritional assessment (MNA)
Social assessment	Income, education, living condition, caregiver

Table 2. Clinical Characteristics of the Subjects

Variables	**Values**
Total number, person	69
Age, yr	73 (69-79)
≥ 75	32 (46.4%)
≥ 80	16 (23.2%)
Gender, female	35 (50.7%)
BMI, kg/m2	25.8 (3.1)
K-FRAIL scale	
Fatigue	14 (20.3%)
Resistance	14 (20.3%)
Ambulation	12 (17.4%)
Illness	1 (1.4%)
Weight loss	2 (2.9%)
Polypharmacy*	36 (52.2%)
History of Herpes Zoster	1 (1.4%)

*Polypharmacy is the concurrent use of 5 more medications.

Table 3. Comparison of demographics, comorbidity, and comprehensive geriatric assessment components of the participants

	All (N =69)	Robust (n=38)	Non-robust[a] (n=31)	*P*
Demographics				
Age (year)	74.3 (5.7)	73.4 (5.8)	75.4 (5.6)	0.150
Age ≥75	32 (46.4%)	15 (39.5%)	17 (54.8%)	0.203
Sex (male/female)	34/35	25/13	9/22	***0.002***
BMI(kg/m^2)	25.8 (3.1)	25.4 (3.1)	26.2 (3.1)	0.366
MAC (cm)	26.3 (2.4)	26.5 (2.0)	26.0 (2.7)	0.638
Calf Circumference (cm)	34.9 (2.8)	35.4 (3.0)	34.3 (2.5)	0.195
Comorbidity				
Hypertension	52 (75.4%)	28 (73.7%)	24 (77.4%)	0.720
Diabetes	12 (17.4%)	8 (21.1%)	4 (12.9%)	0.374
Cardiovascular disease	12 (17.4%)	5 (13.2%)	7 (22.6%)	0.304
Renal disease	5 (7.2%)	0	3 (9.7%)	0.086
Thyroid disease	3 (4.3%)	3 (7.9%)	2 (6.5%)	1.000
Herpes zoster	1 (1.4%)	0	1 (3.2%)	N/A
CGA				
Polypharmacy	36 (52.2%)	18 (47.4%)	18 (58.1%)	0.376
Grip strength (kg)[b]	26.4 (8.7)	29.5 (8.3)	22.5 (7.7)	***0.001***
Low grip strength	13 (18.8%)	4 (10.5%)	9 (29.0%)	0.067
Gait speed (m/s)	1.36 (0.27)	1.46 (0.27)	1.22 (0.20)	***<0.001***
MMSE-KC	25.8 (3.9)	26.8 (3.2)	24.6 (4.3)	***0.004***
SGDS-K	3.1 (3.4)	2.3 (3.2)	4.0 (3.4)	***0.012***
NuDESC	0	0	0	
MNA	26.6 (1.9)	26.8 (1.6)	26.4 (2.3)	0.752

[a]Twenty-nine pre-frail participants and 2 frail participants were included.

[b]The cut-off value for low grip strength in male and female was 28.6 and 16.4 kg, respectively.

CGA, comprehensive geriatric assessment; BMI; body mass index, MAC; mid arm circumference, MMSE-KC, mini-mental status examination (Korean version); SGDS-K, short-form geriatric depression scale (Korean version); NuDESC, nursing delirium screening scale; MNA, mini nutritional assessment; K-FRAIL, fatigue, resistance, ambulation, illnesses, and loss of weight scale-Korean version; N/A; not available.

Table 4. Varicella-zoster virus specific humoral immunongenicity according to frailty status.

Variable	Total participants	Frailty status		*P*-value
	Total	Robust	Non-robust	
ELISA for IgG (IU/mL)	N=69	n =38	n =31	
Baseline GMV	75.59 (39.00–133.42)	79.62 (43.72–148.19)	69.16 (36.10–120.65)	0.398
Post-ZVL GMV	164.38 (112.93–268.97)	166.81 (106.78–266.08)	155.61 (118.36–285.53)	0.736
GMFR	2.27 (1.50–3.46)	2.00 (1.40–3.21)	2.30 (1.67–4.50)	0.210
GMFR >2	37 (53.6%)	19 (50.0%)	18 (58.1%)	0.504

Data are represented as median (interquartile range) or n (percentage).

ZVL, herpes zoster live-attenuated vaccine; ELISA, enzyme-linked immunosorbent assay; GMV, geometric mean value: GMFR, geometric mean fold rise

Table 5. Varicella-zoster virus specific cellular immunogenicity according to frailty status among participants.

Variable	Total participants	Frailty status		*P*-value
	Total	Robust	Non-robust	
IFN-γ ELISPOT (SFCs per 10^6 PBMCs): Baseline SFCs <10				
	N=34	n =20	n =14	
Post-ZVL SFCs ≥10	25 (73.5%)	15 (75.0%)	10 (71.4%)	1.000
IFN-γ ELISPOT (SFCs per 10^6 PBMCs): Baseline SFCs ≥10				
	N=35	n=18	n =17	
Baseline GMV	39.5 (15.0–59.5)	53.3 (21.8–97.6)	24.0 (13.3–42.3)	0.072
Post-ZVL GMV	38.0 (22.0–102.5)	40.8 (23.9–114.9)	38.0 (17.5–93.5)	0.546
GMFR	1.13 (0.46–2.76)	1.09 (0.28–2.89)	1.76 (0.65–2.60)	0.732
GMFR >2	13 (37.1%)	7 (38.9%)	6 (35.3%)	0.826

Data are represented as median (interquartile range) or n (percentage). ZVL, herpes zoster live-attenuated vaccine; IFN-γ ELISPOT, interferon gamma enzyme-linked immunosorbent spot; SFC, spot-forming cell; PBMCs, peripheral blood mononuclear cell; GMV, geometric mean value: GMFR; geometric mean fold rise;

Table 6. Humoral and cellular immunogenicity induced by herpes zoster live-attenuated vaccine according to frailty status.

Variable	**Frailty status**			***P value***		
	1.Robust	2.Pre-frail	3.Frail	1vs.2	2vs.3	1vs.3
ELISA for IgG (IU/mL)	n = 38	n = 29	n = 2			
Baseline GMV	79.62 (43.72–148.19)	75.59 (36.04–127.37)	52.23 (42.65–61.80)	0.494	0.619	0.369
Post-ZVL GMV	166.81 (106.78–266.08)	165.96 (119.66–295.02)	86.43 (49.59–123.27)	0.527	0.108	0.208
GMFR	2.00 (1.40–3.20)	2.36 (1.69–4.88)	1.58 (1.16–1.99)	0.132	0.211	0.400
GMFR > 2	19 (50.0%)	18 (62.1%)	0 (0%)	0.325	N/A	N/A
IFN-γ ELISPOT (SFCs per 106 PBMCs): Baseline SFCs <10						
	n=20	n=12	n=2			
Post SFCs ≥10	15 (75.0%)	9 (75%)	1 (50.0%)	1.000	0.505	0.481
IFN-γ ELISPOT (SFCs per 106 PBMCs): Baseline SFCs ≥10						
	n=18	n=17	n=0			
Baseline GMV	53.3 (21.8–97.6)	24.0 (13.3–42.3)	N/A	0.072	N/A	N/A
Post-ZVL GMV	40.8 (23.8–114.8)	38.0 (17.5–93.5)	N/A	0.546	N/A	N/A
GMFR	1.09 (0.28–2.89)	1.76 (0.65–2.60)	N/A	0.732	N/A	N/A
GMFR > 2	7 (38.9%)	6 (35.3%)	N/A	0.826	N/A	N/A

Data are represented as median (interquartile range) or n (percentage).

ELISA, enzyme-linked immunosorbent assay; ZVL,herpes zoster live-attenuated vaccine; GMV, geometric mean value: GMFR, geometric mean fold rise; PBMCs, peripheral blood mononuclear cells; IFN-γ ELISPOT, interferon gamma enzyme-linked immunosorbent spot; SFC, spot-forming cell; N/A; not available

Table 7. Subgroup analysis of humoral and cellular immunogenicity induced by herpes zoster live-attenuated vaccine and baseline cytokine levels according to frailty status in 65≤age<80.

Variable	Frailty status		*P*
	Robust	Non-robust	
ELISA for IgG (IU/mL)	n=30	n=23	
Baseline GMV	67.35 (33.96–148.19)	75.59 (33.8–135.85)	0.900
Post-ZVL GMV	136.90 (103.91–266.08)	165.96 (115.46–285.53)	0.379
GMFR	2.25 (1.42–3.429)	2.36 (1.61–5.25)	0.351
GMFR > 2	16 (53.3%)	14 (60.9%)	0.583
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs <10			
	n=15	n=11	
Post SFCs ≥10	10 (66.7%)	8 (72.7%)	1.000
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs ≥10			
	n=15	n=12	
Baseline GMV	53.5 (15.0–98.0)	31.8 (15.0–45.9)	0.399
Post-ZVL GMV	36.5 (22.0–125.0)	45.3 (22.6–80.0)	0.943
GMFR	1.08 (0.26–2.98)	1.59 (0.77–2.33)	0.683
GMFR > 2	6 (40.0%)	3 (25.0%)	0.683
Baseline cytokine levels	n=30	n=23	
IL-1β (pg/mL)[a]	0.044 (0.026–0.074)	0.025 (0.008–0.086)	0.420
IL-6 (pg/mL)	0.584 (0.364–0.704)	0.518 (0.395–0.804)	0.844
TNF-α (pg/mL)	2.615 (2.210–3.123)	2.980 (2.260–3.470)	0.311
hsCRP (mg/dL)	0.080 (0.030–0.125)	0.060 (0.030–0.100)	0.589

Data are represented as median (interquartile range) or n (percentage).

[a]Values of 9 participants in the robust group and 5 in non-robust group were in the undetectable range.

ELISA, enzyme-linked immunosorbent assay; ZVL, herpes zoster live-attenuated vaccine; GMV, geometric mean value: GMFR, geometric mean fold rise; PBMCs, peripheral blood mononuclear cells; IFN-γ ELISPOT, interferon gamma enzyme-linked immunosorbent spot; SFC, spot-forming cell; IL-1β; interleukin 1 beta; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; hsCRP, high-sensitivity C-

reactive protein.

Table 8. Subgroup analysis of humoral and cellular immunogenicity induced by herpes zoster live-attenuated vaccine and baseline cytokine levels according to frailty status in age≥85.

Variable	Frailty status		*P*
	Robust	Non-robust	
ELISA for IgG (IU/mL)	n=8	n=8	
Baseline GMV	97.43 (71.87–372.2)	67.13 (37.87–78.35)	0.050
Post-ZVL GMV	187.15 (138.97–621.62)	130.74 (122.21–284.05)	0.328
GMFR	1.76 (1.37–2.55)	2.10 (1.69–3.41)	0.382
GMFR > 2	3 (37.5%)	4 (50.0%)	1.000
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs <10			
	n=5	n=3	
Post SFCs ≥10	5 (100%)	2 (66.7%)	N/A
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs ≥10			
	n=3	n=5	
Baseline GMV	53.0 (47.0–97.5)	23.5 (11.5–30.5)	0.036
Post-ZVL GMV	102 (53.0–111.5)	34.5 (1.25–142.3)	0.786
GMFR	1.13 (1.05–2.10)	2.76 (0.13–4.89)	0.786
GMFR > 2	1 (33.3%)	3 (60.0%)	1.000
Baseline cytokine levels	n=8	n=8	
IL-1β (pg/mL)[a]	0.040 (0.018–0.094)	0.035 (0.011–0.112)	1.000
IL-6 (pg/mL)	0.467 (0.259–0.704)	0.648 (0.538–0.983)	0.105
TNF-α (pg/mL)	2.515 (1.893–3.470)	3.075 (2.795–4.165)	0.195
hsCRP (mg/dL)	0.065 (0.040–0.178)	0.085 (0.055–0.165)	0.442

Data are represented as median (interquartile range) or n (percentage).

[a]Values of 5 participants in the robust group and 4 in non-robust group were in the undetectable range.

ELISA, enzyme-linked immunosorbent assay; ZVL, herpes zoster live-attenuated vaccine; GMV, geometric mean value: GMFR, geometric mean fold rise; PBMCs, peripheral blood mononuclear cells; IFN-γ ELISPOT, interferon gamma enzyme-linked immunosorbent spot; SFC, spot-forming cell; IL-1β; interleukin 1 beta; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; hsCRP, high-sensitivity C-reactive protein; N/A; not available.

Table 9. Humoral and cellular immunogenicity induced by herpes zoster live-attenuated vaccine and baseline cytokine levels according to comorbidity status.

Variable	Comorbidity Status		*P*
	CCI = 0	CCI ≥1	
ELISA for IgG (IU/mL)	n=40	n=29	
Baseline GMV	67.97 (34.47 –142.87)	78.51 (44.71–116.99)	0.584
Post-ZVL GMV	136.90 (112.81–251.85)	195.88 (115.51–353.47)	0.234
GMFR	1.91 (1.42–3.86)	2.49 (1.76–3.33)	0.285
GMFR > 2	19 (47.5%)	18 (62.1%)	0.231
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs <10			
	n=22	n=12	
Post SFCs ≥10	16 (72.7%)	9 (75.0%)	1.000
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs ≥10			
	n=18	n=17	
Baseline GMV	47.5 (20.3–106.4)	35.0 (13.3–50.0)	0.143
Post-ZVL GMV	35.5 (20.3–110.8)	52.5 (24.5–102.3)	0.708
GMFR	0.99 (0.26–2.78)	1.41 (0.89–2.91)	0.219
GMFR > 2	5 (27.8%)	8 (47.1%)	0.238
Baseline cytokine levels	n=40	n=29	
IL-1β (pg/mL)[a]	0.044 (0.020–0.083)	0.028 (0.019–0.072)	0.794
IL-6 (pg/mL)	0.522 (0.394–0.635)	0.676 (0.417–0.862)	***0.038***
TNF-α (pg/mL)	2.615 (2.188–3.070)	3.050 (2.645–3.995)	***0.005***
hsCRP (mg/dL)	0.050 (0.030–0.090)	0.090 (0.060–0.175)	***0.010***

Data are represented as mean (standard deviation) or n (percentage).

[a]Data of 11 participants of CCI=0 group and 12 participants of CCI≥1 group were unavailable.

ZVL, herpes zoster live-attenuated vaccine; CCI, Charlson Comorbidity Index; ELISA, enzyme-linked immunosorbent assay; GMV, geometric mean value: GMFR, geometric mean fold rise; IFN-γ ELISPOT, interferon gamma enzyme-linked immunosorbent spot; SFC, spot-forming cell; PBMCs,

peripheral blood mononuclear cells; IL-1β , interleukin 1-beta; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha; hsCRP, high sensitivity C-reactive protein.

Table 10. Humoral and cellular immunogenicity induced by herpes zoster live-attenuated vaccine and baseline cytokine levels according to mid-arm circumference.

Variable	Mid-arm circumference		*P*
	MAC > 27	MAC ≤ 27	
ELISA for IgG (IU/mL)	n=24	n=45	
Baseline GMV	71.16 (43.20–116.22)	75.59 (36.19–148.56)	0.588
Post-ZVL GMV	188.82 (107.26–297.06)	149.45 (113.73–257.81)	0.696
GMFR	2.39 (1.91–5.10)	1.89 (1.43–3.23)	0.118
GMFR > 2	16 (66.7%)	21 (46.7%)	0.113
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs <10			
	n=13	n=21	
Post SFCs ≥10	9 (69.2%)	16 (76.2%)	0.704
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs ≥10			
	n=11	n=14	
Baseline GMV	25.0 (18.0–98.0)	42.3 (14.3–58.0)	0.930
Post-ZVL GMV	25.5 (13.0–118.0)	48.8 (25.3–102.4)	0.370
GMFR	0.72 (0.26–2.98)	1.59 (0.79–2.68)	0.409
GMFR > 2	4 (36.4%)	9 (37.5%)	1.000
Baseline cytokine levels	n=24	n=45	
IL-1β (pg/mL)[a]	0.027 (0.007–0.084)	0.038 (0.022–0.084)	0.280
IL-6 (pg/mL)	0.641 (0.451–0.900)	0.526 (0.374–0.683)	0.061
TNF-α (pg/mL)	2.905 (2.258–3.465)	2.860 (2.195–3.165)	0.484
hsCRP (mg/dL)	0.065 (0.033–0.143)	0.070 (0.040–0.110)	0.841

Data are represented as mean (standard deviation) or n (percentage).

[a]Data of 6 participants of MAC>27 group and 17 participants of MAC≤27 group were unavailable.

ZVL, herpes zoster live-attenuated vaccine; CCI, Charlson Comorbidity Index; ELISA, enzyme-linked immunosorbent assay; GMV, geometric mean value: GMFR, geometric mean fold rise; IFN-γ ELISPOT, interferon gamma enzyme-linked immunosorbent spot; SFC, spot-forming cell; PBMCs, peripheral blood mononuclear cells; IL-1β , interleukin 1-beta; IL-6, interleukin 6; TNF-α, tumour

necrosis factor alpha; hsCRP, high sensitivity C-reactive protein.

Table 11. Humoral and cellular immunogenicity induced by herpes zoster live-attenuated vaccine and baseline cytokine levels according to age group

Variable	Age group		*P*
	Age ≤65 < 80	Age ≥80	
ELISA for IgG (IU/mL)	n=53	n=16	
Baseline GMV	71.17 (33.93–137.92)	77.97 (62.41–124.71)	0.410
Post-ZVL GMV	155.61 (107.71–268.97)	171.40 (126.97–287.56)	0.550
GMFR	2.36 (1.50–3.77)	1.91 (1.45–2.82)	0.378
GMFR > 2	30 (56.6%)	7 (43.8%)	0.366
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs <10			
	n=26	n=8	
Post SFCs ≥10	18 (69.2%)	7 (87.5%)	0.403
IFN-γ ELISPOT (SFs per 106 PBMCs): Baseline SFCs ≥10			
	n=27	n=8	
Baseline GMV	42.0 (15.0–96.5)	30.5 (15.3–51.5)	0.524
Post-ZVL GMV	36.5 (22.0–97.5)	77.5 (10.5–116.4)	0.576
GMFR	1.09 (0.46–2.44)	1.61 (0.44–3.22)	0.743
GMFR > 2	9 (33.3%)	4 (50.0%)	0.433
Baseline cytokine levels	n=53	n=16	
IL-1β (pg/mL)[a]	0.033 (0.020–0.080)	0.040 (0.018–0.094)	0.976
IL-6 (pg/mL)	0.581 (0.374–0.755)	0.553 (0.459–0.751)	0.675
TNF-α (pg/mL)	2.880 (2.225–3.165)	2.855 (2.325–3.768)	0.451
hsCRP (mg/dL)	0.060 (0.030–0.110)	0.070 (0.050–0.165)	0.275

Data are represented as median (interquartile range) or n (percentage).

[a]Values of 14 participants in the <80 year age group and 9 in the ≥80 age group were in the undetectable range.

ELISA, enzyme-linked immunosorbent assay; ZVL, herpes zoster live-attenuated vaccine; GMV, geometric mean value: GMFR, geometric mean fold rise; PBMCs, peripheral blood mononuclear cells; IFN-γ ELISPOT, interferon gamma enzyme-linked immunosorbent spot; SFC, spot-forming cell; IL-1β; interleukin 1 beta; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; hsCRP, high-sensitivity C-reactive protein.

Table 12. Baseline cytokine levels according to frailty status

Variable	Total participants	Frailty status		*P*
	Total	Robust	Non-robust	
cytokine level	N=69	n=38	n=31	
IL-1β (pg/mL) [a]	0.034	0.042	0.025	0.253
	(0.019–0.082)	(0.025–0.077)	(0.008–0.086)	
IL-6 (pg/mL)	0.577	0.545	0.588	0.341
	(0.379–0.760)	(0.364–0.704)	(0.431–0.804)	
TNF-α (pg/mL)	2.860	2.615	2.980	0.087
	(2.225–3.295)	(2.163–3.163)	(2.570–3.520)	
hsCRP (mg/dL)	0.070	0.075	0.070	0.937
	(0.040–0.115)	(0.038–0.123)	(0.040–0.110)	

Data are represented as median (interquartile range)

[a]Data of 14 non-frail participants and 9 'prefrail-to-frail' participants were unavailable.

IL-1β , interleukin 1-beta; IL-6, interleukin 6; TNF-α, tumour necrosis factor alpha; hsCRP, high sensitivity C-reactive protein.

Figures

Figure 1. Schematic overview of Enzyme-linked Immunosorbent Assay (ELISA) to detect Varicella-Zoster Virus specific IgG antibody

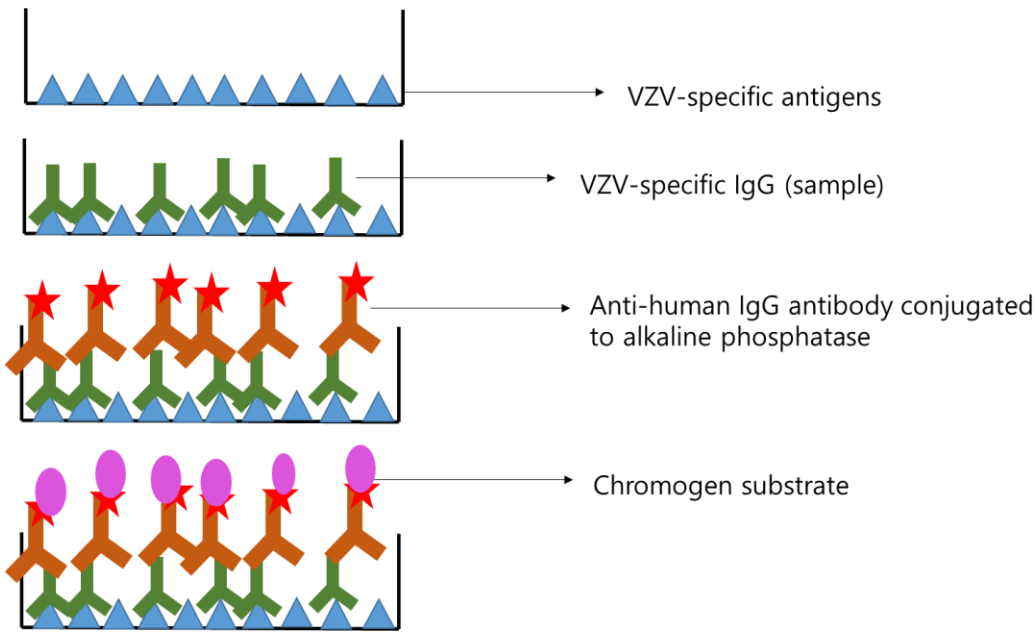


Figure 2. Schematic overview of IFN-γ enzyme-linked immunosorbent spot assay

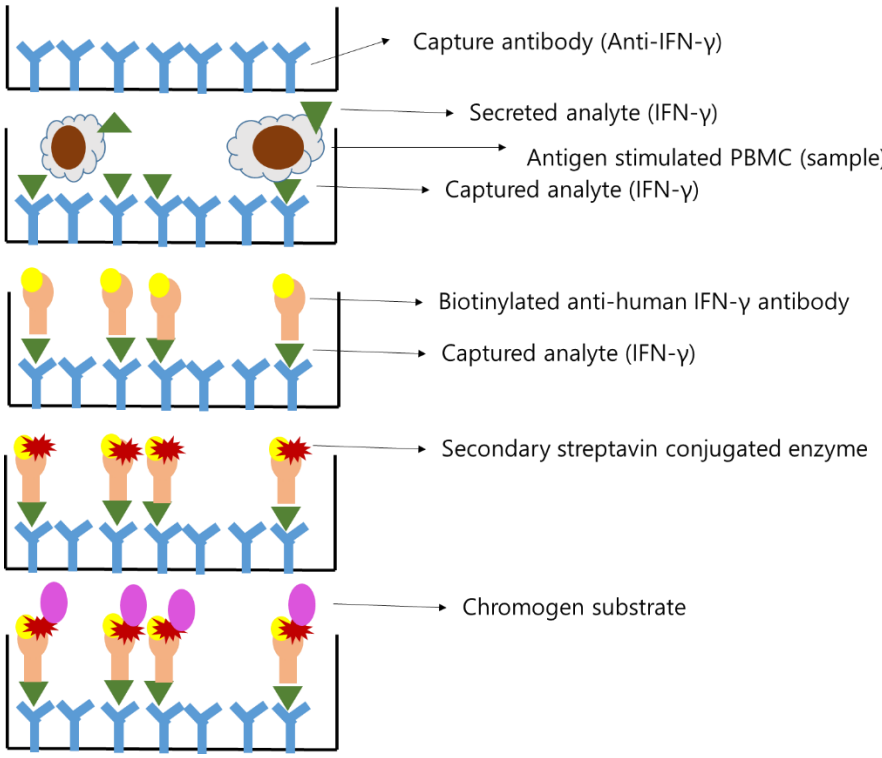
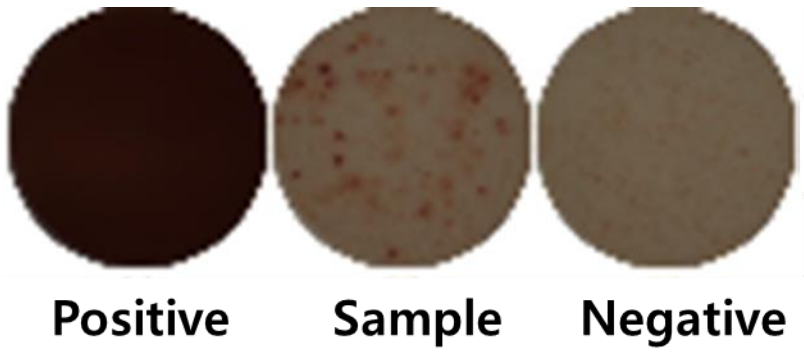


Figure 3. Example of spot forming cells (SFCs) following development of IFN-γ enzyme-linked immunosorbent assay



The negative control antigen was produced via the same process using uninfected MRC-5 cells. And the phytohemagglutinin (PHA) was incorporated as a positive control

Figure 4. Schematic overview of Enzyme-linked Immunosorbent Assay (ELISA) to analyze cytokine

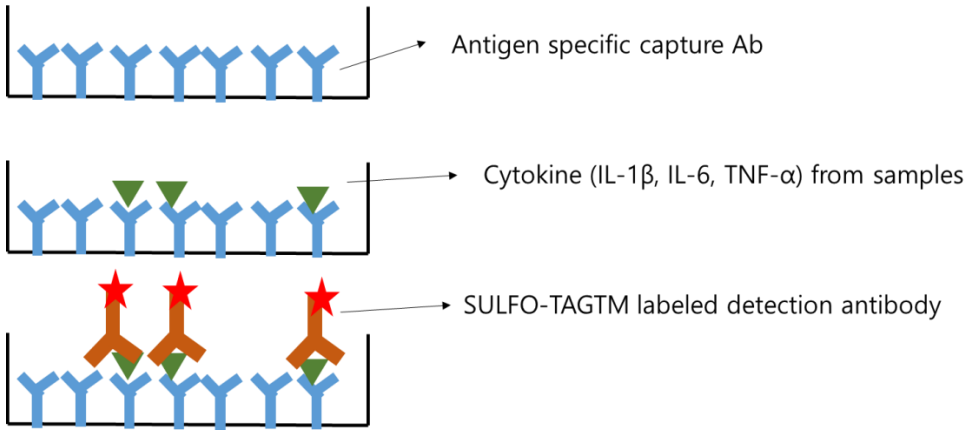


Figure 5. Enrollment of the participants

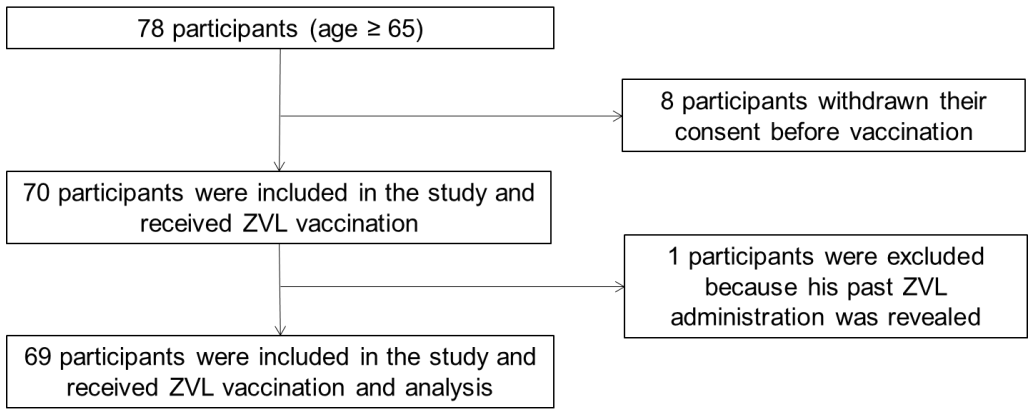


Figure 6. ELISA level of pre vaccination and post 6 weeks after vaccination according to frailtystatus

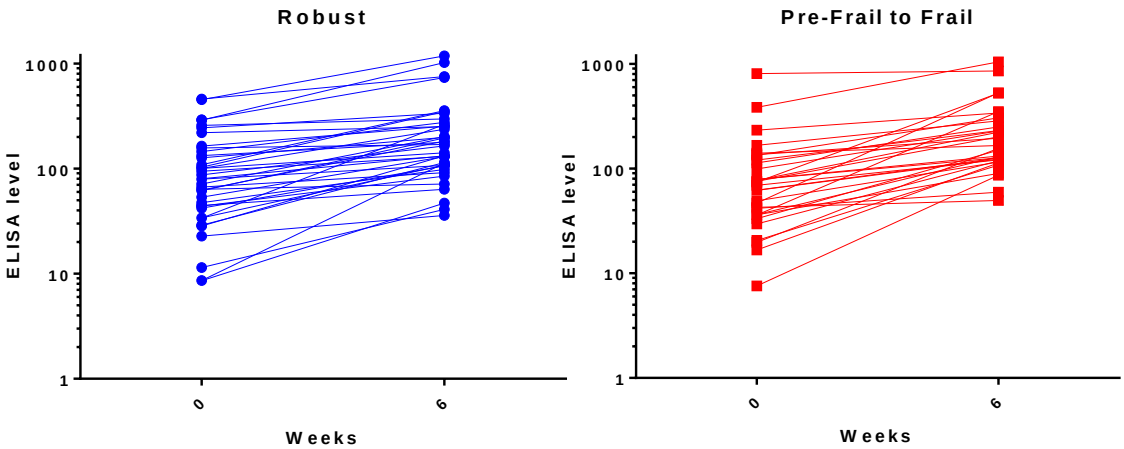


Figure 7. ELISPOT level of pre vaccination and post 6 weeks after vaccination according to frailty status

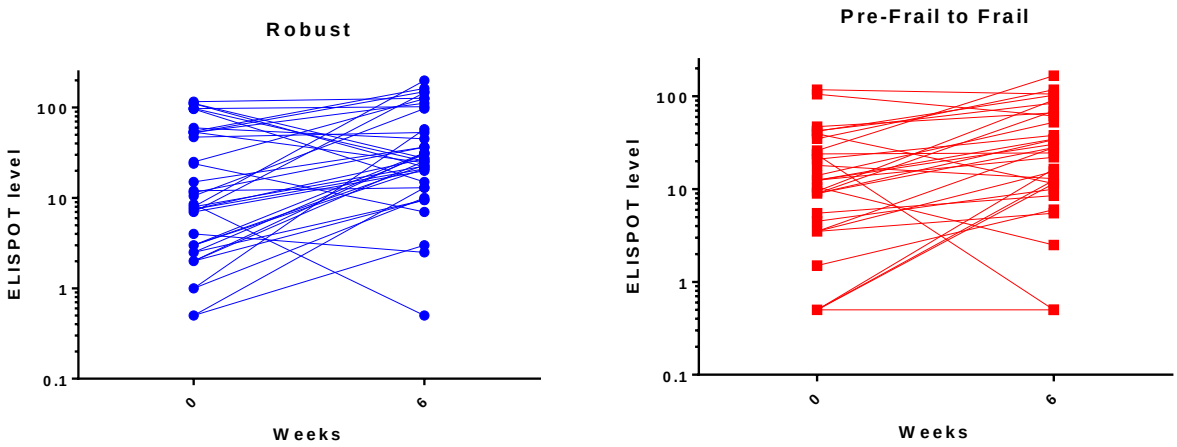


Figure 8-A. Baseline IL-1ß levels of all participants

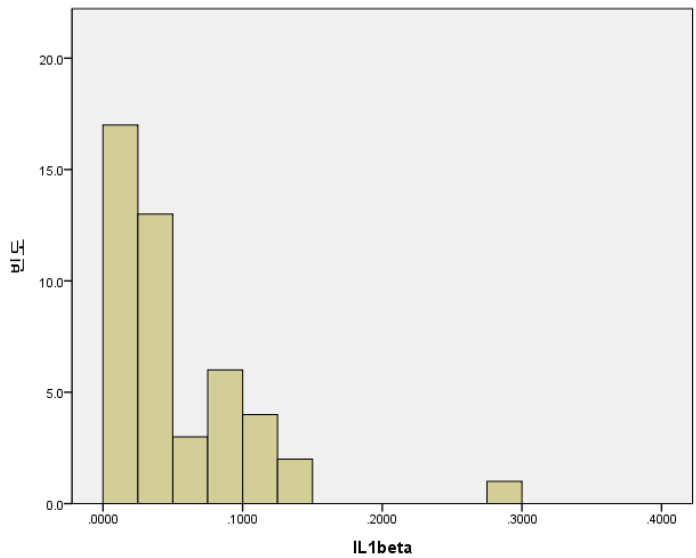


Figure 8-B. Baseline IL-6 levels of all participants

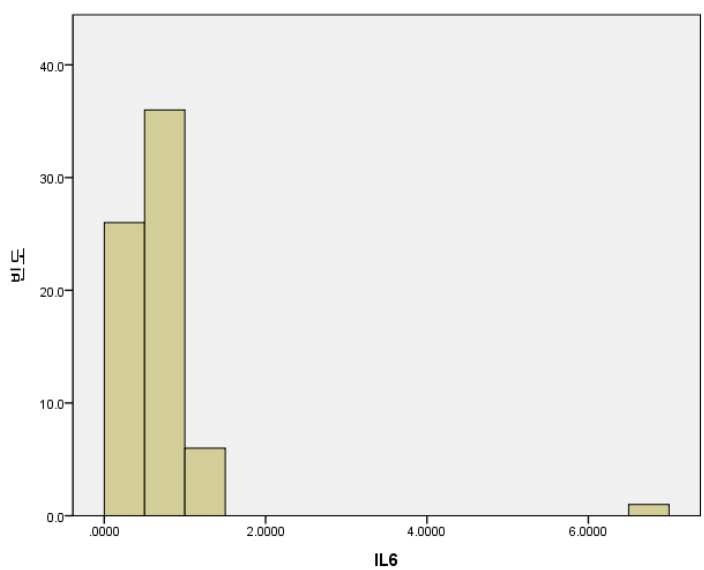


Figure 8-C. Baseline TNF-α levels of all participants

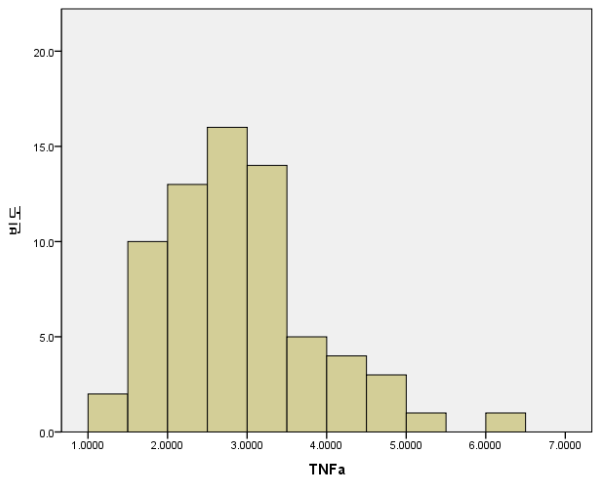
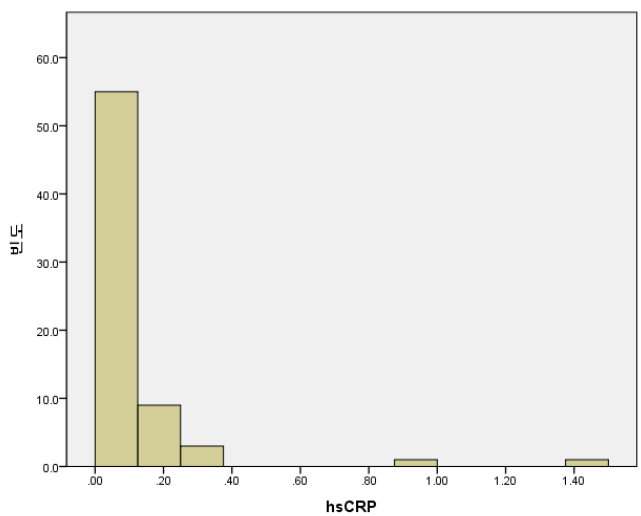
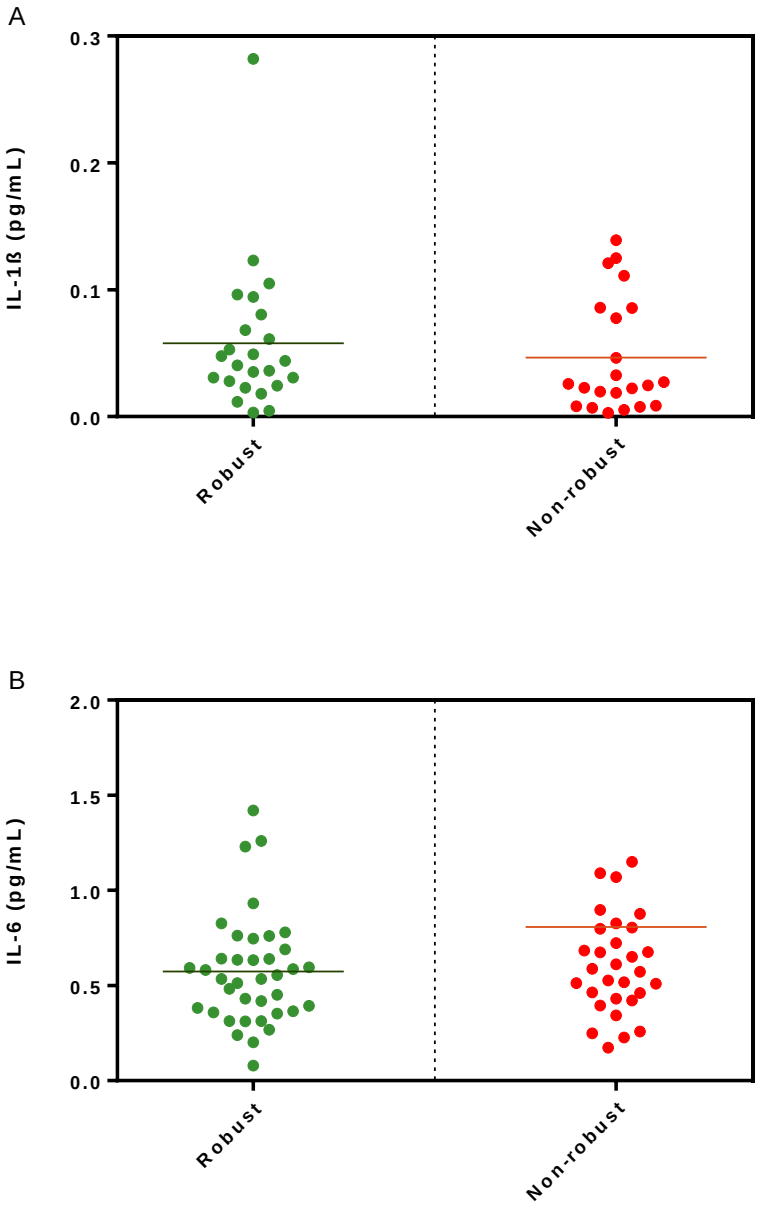


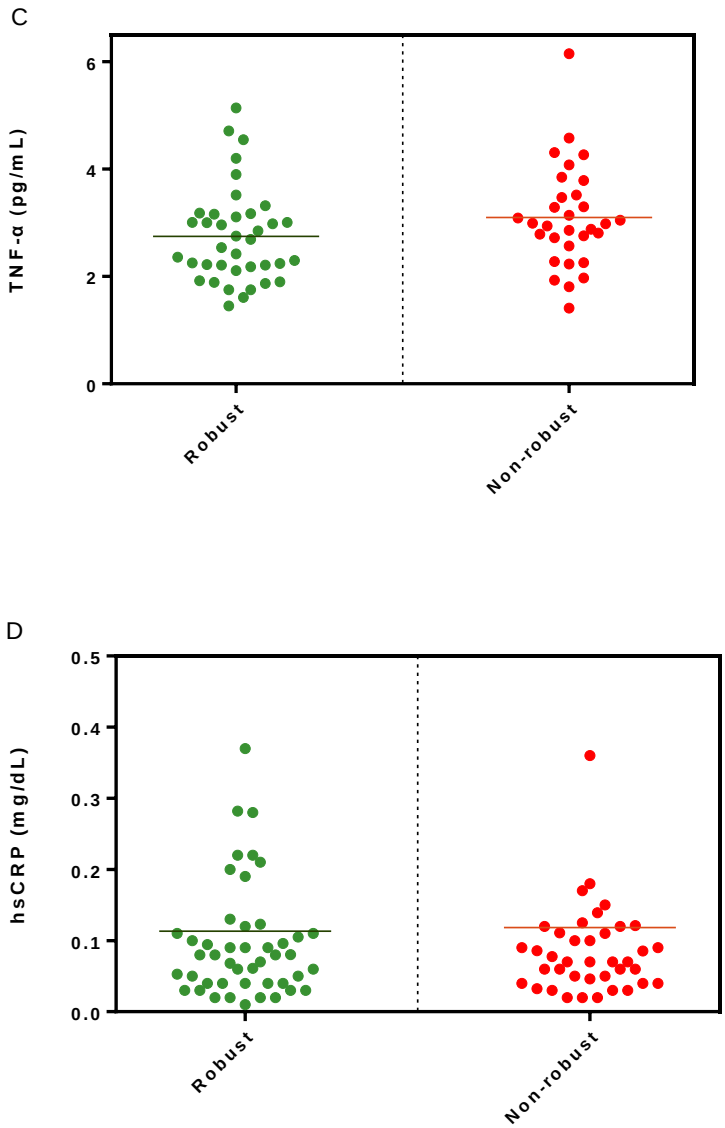
Figure 8-D. Baseline hsCRP levels of all participants



IL-1β levels or 21 participants were undetectable.

Figure 9. Baseline inflammatory marker levels according to frailty status. (A,B,C,D for IL-1β, IL-6, TNF- α and hsCRP)

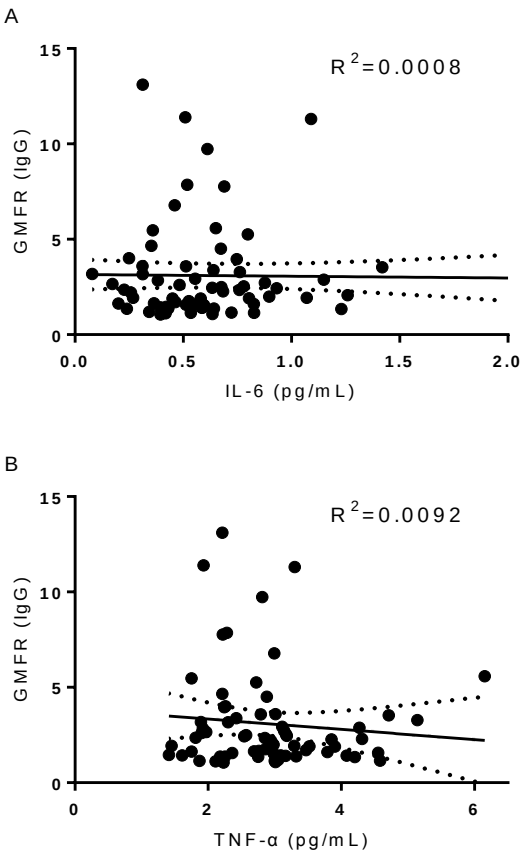


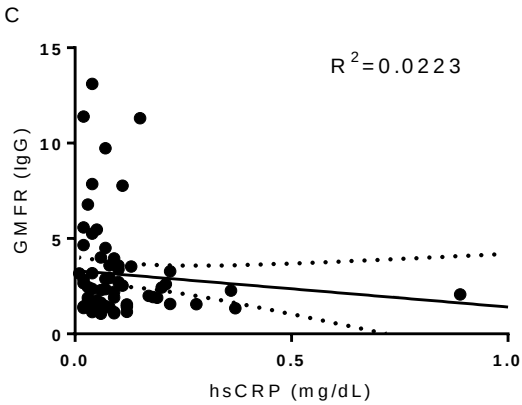


IL-1β levels or 14 robust participants and 9 non-robust participants were undetectable.

One participant in the non-robust group with an IL-6 level 6.88 pg/mL is outside the plotted area in panel B.
One participant from the robust group with hsCRP levels of 0.89 mg/dL and another from the non-robust group with an hsCRP level of 1.43 mg/dL are outside the plotted area in panel D.
IL-1β, interleukin 1 beta; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; hsCRP, high sensitivity C-reactive protein.

Figure 10. Scatter plots of baseline levels of IL-6, TNF-α, and hsCRP with humoral immunogenicity.



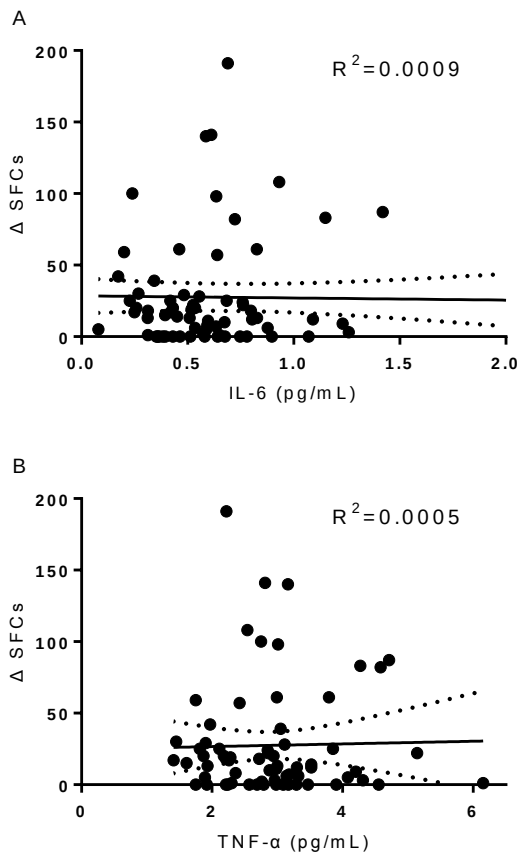


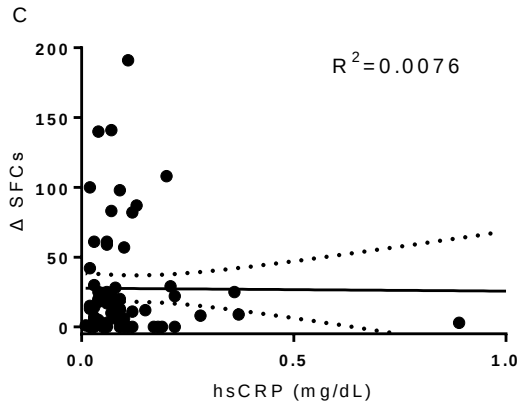
One participant (IL-6 level of 6.88 pg/mL) of figure A and one participant (hsCRP level of 1.43 mg/dL) of figure C are out of the graph.

Thick line indicates mean trend line and dotted line indicates 95% confidence interval.

GMFR, geometric mean fold rise; IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; hsCRP, high-sensitivity C-reactive protein.

Figure 11. Scatter plots of baseline IL-6 and TNF-α and hsCRP levels with cellular immunogenicity





One participant with an IL-6 level of 6.88 pg/mL in panel A and 1 participant with an hsCRP level of 1.43 mg/dL in panel C are outside the plot area.

The thick line indicates the mean trendline while the dotted line represents the 95% confidence interval.

ΔSFCs indicates difference between SFCs before and 6-weeks after herpes zoster live-attenuated vaccination (SFCs/per 10^6 cells).

IL-6, interleukin 6; TNF-α, tumor necrosis factor alpha; hsCRP, high-sensitivity C-reactive protein; SFC, spot-forming cell.

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