This is the accepted manuscript of the article, which has been published in *Human Vaccines and Immunotherapeutics*. 2019. https://doi.org/10.1080/21645515.2019.1627160

Antibody response to the 23-valent pneumococcal polysaccharide vaccine after conjugate vaccine in patients with chronic lymphocytic leukemia

Running title: Antibody response to PPV23 booster in patients with CLL

Corresponding author:

Vesa Lindström, MD

Department of Hematology

Comprehensive Cancer Center

Helsinki University Hospital

Haartmaninkatu 4, P.O.Box 372, 00029 HUS, Helsinki Finland

vesa.lindstrom@hus.fi

phone: +358504279011

ORCID 0000-0001-5724-9120

Co-authors:

Janne Aittoniemi, MD, PhD

Department of Clinical Microbiology

Fimlab Laboratories

P.O.Box 66, 33101, Tampere Finland

janne.aittoniemi@fimlab.fi

+358331177800

Urpu Salmenniemi, MD, PhD

Department of Hematology and Stem Cell Transplantation Unit

Division of Medicine

Turku University Hospital

P.O.Box 52, 20521 Turku, Finland

urpu.salmenniemi@tyks.fi

+35823130000

Helena Käyhty, Research prof emerita, visiting scientist

Department of Health Security

National Institute for Health and Welfare

P.O.Box 30, 00271 Helsinki, Finland

helena.kayhty@thl.fi

+3580295246000

Heini Huhtala, MSc

Faculty of Social Sciences

University of Tampere

P.O.Box 100, 33014 Tampere, Finland

heini.huhtala@tuni.fi

+358401901663

Marjatta Sinisalo, MD, PhD

Department of Internal Medicine

Tampere University Hospital

P.O.Box 2000, 33521 Tampere, Finland

marjatta.sinisalo@pshp.fi

+3583311611

Abstract

The 23-valent pneumococcal polysaccharide vaccine (PPV23) given alone is ineffective in patients with chronic lymphocytic leukemia (CLL) and better antibody response is achieved with pneumococcal conjugate vaccines (PCVs). In this study we determine whether CLL patients would achieve a significant antibody response and broaden their serotype coverage against invasive pneumococcal disease (IPD) with PPV23 given five years after the 7-valent conjugate vaccine (PCV7). A total of 24 patients with CLL and eight controls were vaccinated with PPV23 five years after PCV7. Blood samples for evaluation of antibody response to PCV7 serotypes and PPV23 serotypes 5 and 7 were taken before vaccination and one month after it. Post-vaccination samples were available from 20 patients. IgG antibodies were measured with ELISA. Antibody concentrations after PPV23 were significantly lower in CLL patients for six of the PCV7 serotypes and for both PPV23 serotypes. Only 10 to 15% of CLL patients achieved an antibody response suggested to be protective against IPD. Hence, PCV7 given five years before PPV23 did not improve antibody response in patients with CLL. Based on our results, PPV23 given after a PCV primer is not useful against IPD in CLL patients.

Keywords: chronic lymphocytic leukemia; pneumococcal polysaccharide vaccine; pneumococcal conjugate vaccine; booster vaccination; antibody response

Introduction

Infections are the most important cause of mortality in patients with chronic lymphocytic leukemia (CLL), the most common type of leukemia in adult Caucasians. The usual bacterial pathogens responsible for respiratory tract and urinary tract infections are *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Escherichia coli*.[1-2] CLL is associated with impaired immune system function even at an early stage of the disease.[3] Both the specific and innate immunity are affected, leading to immunosuppression in patients with CLL.[4] Abnormalities in specific (adaptive) immunity includes B- and T-cell dysfunction while disorders of complement system and neutrophil functions represent abnormalities in innate immunity.[1,4,5-6] While hypogammaglobulinemia can occur at an early stage of CLL, it is usually more severe in the advanced stages of the disease.[7] Low levels of IgG have been observed to correlate with severity of bacterial infections, morbidity, and mortality.[8]

In contrast to pneumococcal polysaccharide vaccine (PPV), the T-cell dependent pneumococcal conjugate vaccines (PCVs) have been shown to induce higher avidity antibodies and immunologic memory and to create herd immunity.[9] Antibody responses to pneumococcal vaccines in CLL patients are limited. Most studies with 23-valent PPV (PPV23) have shown no significant antibody responses.[10-13] PCVs, in contrast, have proved immunogenic in CLL patients with limited response in a few earlier studies. In almost 40% of CLL patients, a response, that was considered significant was achieved after one dose of 7-valent PCV (PCV7) given at an early stage of the disease.[14] Furthermore, 13-valent PCV (PCV13) resulted in at least a two-fold increase in antibody titers from the baseline in 58% of untreated CLL patients, compared with 100% for healthy controls.[15] In a recent randomized study PCV13 was compared to PPV23 in untreated CLL patients. Functional antibody titers measured by opsonophagocytic assay (OPA) were significantly higher for 11 out of

13 serotypes with PCV13 at one month after vaccination and for 6 out of 13 serotypes at six months after vaccination compared to PPV23. All antibody responses for PPV23 were inferior than achieved with PCV13.[16]

PPV23 given after PCV may have a booster effect on antibody responses and may also broaden serotype coverage to PPV23 serotypes in immunocompromised patients. Patients with previously treated Hodgkin lymphoma achieved higher antibody concentrations for six serotypes contained in both vaccines when PPV23 was given one year after PCV7 in contrast to PPV23 alone.[17] In stem cell transplant (SCT) recipients, the antibody response rates to pneumococcal serotypes 1 and 5 not included in PCV7 were 83% after one dose of PPV23 at 12 months and 89% 18 months after 3 doses of PCV7 with the cut-off of ≥0.15 μg/ml set for response.[18] Furthermore, 42% of allogeneic SCT recipients, who did not show a response after three doses of PCV7 achieved an antibody response of ≥0.15 μg/ml to the PCV7 antigens after a single dose of PPV23.[19]

In this study we investigated the effect of a single dose of PPV23 given five years after PCV7 to evaluate whether CLL patients could obtain a boost to their antibody response and broaden their serotype coverage against invasive pneumococcal disease (IPD) with PPV23 primed with PCV7.

Patients and methods

The study population comprised 24 patients with CLL (12 males and 12 females), with a median age of 64 years (range 47-86 years) from Tampere and Turku University Hospitals. The control population comprised eight subjects (median age 67 years, range 57-82 years, four males and four females) without any known immunological or hematological defects from Tampere University Hospital. The patients and control subjects had participated in an earlier pneumococcal conjugate

vaccine response study with PCV7 containing capsular polysaccharides (PSs) of pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F, and in an antibody persistence study.[14,20]

An informed consent to participate was obtained from all patients and controls. The study was approved by the ethical board of the Pirkanmaa Hospital District and it was conducted in accordance with the Declaration of Helsinki. The trial was registered at *http://ClinicalTrials.gov (NCT00919321).*

The study objectives were to compare antibody concentrations for PCV7 antigens and PPV23 antigens 5 and 7F, as an example for antigens which are not included in PCV7, at one month after vaccination in CLL patients and controls with serotype-specific IgG antibodies as measured by ELISA and to determine the proportions of CLL patients achieving a significant serotype-specific antibody response against IPD after PPV23.

The vaccine used was the 23-valent pneumococcal polysaccharide vaccine (Pneumovax®, MSD), which contains capsular PSs of pneumococcal serotypes 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19F, 19A, 20, 22F, 23F, 33F. A dose of 0.5 ml contains 25 µg of each PS type and phenol as an adjuvant.

Patients and controls received one intramuscular deltoid injection with Pneumovax five years after PCV7. Venous blood samples for antibody analyses were taken before vaccination and one month after it. Serum was separated by centrifugation and stored at -20°C. The concentrations of serum IgG antibody against pneumococcal capsular PSs were measured at the National Institute for Health and Welfare (THL) by a modification of the 22F inhibition enzyme immunoassay (EIA) method as previously described.[21] Additionally, venous blood samples were drawn from CLL patients for the

analysis of total blood count and immunoglobulins at the time of vaccination. These were analyzed at a local hospital.

Antibody concentrations and proportionate changes between groups were compared by independent-samples Mann-Whitney test and the significance of antibody responses within groups by 2-tailed Fisher´s exact test. The values in the tables are expressed as geometric mean concentrations (GMC) and quartiles. An antibody concentration of 0.35 µg/ml was considered as a threshold for protection against IPD, as recommended by the World Health Organization WHO.[22] A significant antibody response was defined as an at least two-fold increase from the baseline and a post-vaccination level of at least 0.35 µg/ml consistent with earlier PCV7 study.[14]

Results

Among the 24 CLL patients and eight controls enrolled in the study, post-vaccination samples were unavailable in the case of four patients. Therefore, the final study population comprised 20 CLL patients and eight controls (Table 1). No vaccine-related adverse events were reported. The disease status according to Binet classification was A (early stage of the disease) in 12, B (intermediate) in two, and C (advanced stage) in six patients. A total of 13 patients had never been treated for CLL. After administration of PCV7, six patients had received chemoimmunotherapy or chemotherapy, one patient CD52 monoclonal antibody (alemtuzumab) after chemotherapy and one patient had received allogeneic stem cell transplantation. Hypogammaglobulinemia (S-IgG <6.77 g/l) was detected in nine (45%) patients. Five patients had suffered from severe infections (needing intravenous antibiotics or hospitalization) and five patients from mild to moderate infections (treated with oral antibiotics) during the five years since PCV7 vaccination. Only one of these infections was invasive pneumococcal disease.

Antibody concentrations after PPV23 were significantly higher in controls for four of PCV7 antigens (4, 9V, 14, 18C) and for both PPV23 antigens (Table 2). In contrast, no difference was observed in antibody concentrations for PCV7 antigens 6B, 19F and 23F. Before PPV23, i.e. five years after PCV7, no statistically significant differences were seen in antibody concentrations between patients with CLL and controls.[20]

After PPV23, only 10 to 15% of CLL patients achieved a significant response to PCV7 antigens defined as an at least two-fold increase and a post-vaccination concentration of at least 0.35 µg/ml (Table 3). Among CLL patients, the responders were the same four patients depending on the serotype and they had never been treated for CLL. These patients were also among the PCV7 responders. For PPV23 antigens 5 and 7, the corresponding percentages for CLL patients were 20% and 15%, respectively. Despite of poor antibody response, 30-75% of CLL patients remained their antibody concentrations at a level ≥0.35 µg/ml suggested to be protective against IPD depending on serotype. In controls, 75-88% achieved a significant response to PCV7 antigens 4, 6B, 9V, 14, 18C, 19F. In contrast, the corresponding rate for serotype 23F was only 50%. For PPV23 antigens, all controls achieved a significant response.

Discussion

According to a few earlier studies, patients with Hodgkin´s lymphoma and SCT recipients benefit from a PCV primer before PPV23.[17-19] Furthermore, in HIV-infected adults, PPV23 given one year after PCV13 improved PCV13 immunogenicity but in contrast had a negative effect on immunological memory induced by PCV13 by reducing the amounts of memory B-cells.[23] Similar findings have been reported where PPV23 given after PCV depletes immunological memory. Longer intervals between pneumococcal vaccinations diminish this hyporesponsiveness and are

beneficial for immunogenicity.[24] Our study was designed based on a long interval between vaccinations consistent with an updated recommendation.[25] However, we did not observe this booster effect in CLL patients. Furthermore, a majority of the untreated CLL patients did not achieve an antibody response considered as significant for PPV23. Hence, in our data CLL therapy after PCV7 did not seem to have an influence on poor antibody responses for PPV23.

An antibody concentration of 1.3 µg/ml is recommended as cut-off for protective antibody level against IPD in primary immunodeficiencies.[26] This higher cut-off level may also be extrapolated to acquired immunodeficiencies, such as CLL related immune dysfunction. In our data, all responders in CLL group achieved this higher antibody concentration expect two out of four CLL patients for antibody 5.

This is the first study to evaluate the effect of the 23-valent polysaccharide vaccine after the conjugate vaccine (PCV7) in patients with CLL. Although ≥50% of CLL patients remained their antibody concentrations at a protective level for 7 out of 9 serotypes, most of the CLL patients did not achieve an antibody response considered to be significant, as compared to controls. These data suggest that PCV is not capable of inducing memory B-cells in CLL patients, although our earlier data showed antibody persistence at least five years after PCV7, depending on the serotype.[20] One probable reason for a poor antibody response with PPV23 after the PCV primer is abnormalities in both B- and T –cell functions even at an early stage of CLL, leading to inadequate immunogenicity of conjugate vaccines.[1,4,15] Stage of the disease in our data did not impair antibody responses as most of the patients were Binet A patients.

Our study has some limitations. The sample size remained small due to the natural course of CLL and the five-year interval between vaccinations. PCV7 has also been replaced with PCV13, but both

have the same carrier protein, CRM.[9] Furthermore, PCV13 has also been shown to generate suboptimal responses in CLL patients.[15-16] Hence, our data should reflect immune responses to both PCV7 and PCV13.

A PPV23 booster dose given five years after PCV7 seemed to be ineffective in inducing an antibody response suggested to be protective against IPD in CLL patients. Based on these data, PPV23 may not be useful as a part of the vaccination program after PCVs in patients with CLL. Future studies with the PCV13 primer are warranted in order to verify our data. Furthermore, whether CLL patients would benefit from a PCV13 booster instead of PPV23 needs to be studied.

Disclosure of potential conflicts of interest

The authors declare no conflicts of interest.

References

1. Morrison VA. Infectious complications of chronic lymphocytic leukaemia: Pathogenesis, spectrum of infection, preventive approaches. Best Practice & Research Clinical Haematology. 2010;23:145-153.

2. Linet MS, Schubauer-Berigan MK, Weisenburger DD, Richardson DB, Landgren O, Blair A, Silver S, Field RW, Caldwell G, Hatch M, et al. Chronic lymphocytic leukaemia: An overview of aetiology in light of recent developments in classification and pathogenesis. Br J Haematol. 2007;139:672-686.

3. Moreira J, Rabe KG, Cerhan JR, Kay NE, Wilson JW, Call TG, Leis JF, Jelinek DF, Schwager SM, Bowen DA, et al. Infectious complications among individuals with clinical monoclonal B-cell lymphocytosis (MBL): A cohort study of newly diagnosed cases compared to controls. Leukemia. 2013;27:136-141.

4. Dearden C. Disease-specific complications of chronic lymphocytic leukemia. ASH Education Program Book. 2008;2008:450-456.

5. Schlesinger M, Broman I, Lugassy G. The complement system is defective in chronic lymphatic leukemia patients and in their healthy relatives. Leukemia. 1996;10:1509-1513.

6. Kontoyiannis DP, Georgiadou SP, Wierda WG, Wright S, Albert ND, Ferrajoli A, Keating M, Lewis RE. Impaired bactericidal but not fungicidal activity of polymorphonuclear neutrophils in patients with chronic lymphocytic leukemia. Leuk Lymphoma. 2013;54:1730-1733.

7. Forconi F, Moss P. Perturbation of the normal immune system in patients with CLL. Blood. 2015;126:573-581.

8. Rozman C, Montserrat E, Vinolas N. Serum immunoglobulins in B-chronic lymphocytic leukemia. natural history and prognostic significance. Cancer. 1988;61:279-283.

9. Pichichero ME. Protein carriers of conjugate vaccines: Characteristics, development, and clinical trials. Hum Vaccin Immunother. 2013;9:2505-2523.

10. Sinisalo M, Aittoniemi J, Oivanen P, Käyhty H, Ölander R, Vilpo J. Response to vaccination against different types of antigens in patients with chronic lymphocytic leukaemia. Br J Haematol. 2001;114:107-110.

11. Hartkamp A, Mulder AH, Rijkers GT, van Velzen-Blad H, Biesma DH. Antibody responses to pneumococcal and haemophilus vaccinations in patients with B-cell chronic lymphocytic leukaemia. Vaccine. 2001;19:1671-1677.

12. Safdar A, Rodriguez GH, Rueda AM, Wierda WG, Ferrajoli A, Musher DM, O'Brien S, Koller CA, Bodey GP, Keating MJ. Multiple-dose granulocyte-macrophage-colony-stimulating factor plus 23-valent polysaccharide pneumococcal vaccine in patients with chronic lymphocytic leukemia: A prospective, randomized trial of safety and immunogenicity. Cancer. 2008;113:383-387.

13. Van der Velden AM, Van Velzen-Blad H, Claessen AM, Van der Griend R, Oltmans R, Rijkers GT, Biesma DH. The effect of ranitidine on antibody responses to polysaccharide vaccines in patients with B-cell chronic lymphocytic leukaemia. Eur J Haematol. 2007;79:47-52.

14. Sinisalo M, Vilpo J, Itälä M, Väkeväinen M, Taurio J, Aittoniemi J. Antibody response to 7-valent conjugated pneumococcal vaccine in patients with chronic lymphocytic leukaemia. Vaccine. 2007;26:82-87.

15. Pasiarski M, Rolinski J, Grywalska E, Stelmach-Goldys A, Korona-Glowniak I, Gozdz S, Hus I, Malm A. Antibody and plasmablast response to 13-valent pneumococcal conjugate vaccine in chronic lymphocytic leukemia patients--preliminary report. PLoS One. 2014;9:e114966.

16. Svensson T, Kättström M, Hammarlund Y, Roth D, Andersson PO, Svensson M, Nilsson I, Rombo L, Cherif H, Kimby E. Pneumococcal conjugate vaccine triggers a better immune response than pneumococcal polysaccharide vaccine in patients with chronic lymphocytic leukemia A randomized study by the swedish CLL group. Vaccine. 2018;36:3701-3707.

17. Chan CY, Molrine DC, George S, Tarbell NJ, Mauch P, Diller L, Shamberger RC, Phillips NR, Goorin A, Ambrosino DM. Pneumococcal conjugate vaccine primes for antibody responses to polysaccharide pneumococcal vaccine after treatment of Hodgkin's disease. J Infect Dis. 1996;173:256-258.

18. Cordonnier C, Labopin M, Chesnel V, Ribaud P, Camara Rde L, Martino R, Ullmann AJ, Parkkali T, Locasciulli A, Yakouben K, et al. Immune response to the 23-valent polysaccharide pneumococcal vaccine after the 7-valent conjugate vaccine in allogeneic stem cell transplant recipients: Results from the EBMT IDWP01 trial. Vaccine. 2010;28:2730-2734.

19. Cordonnier C, Labopin M, Chesnel V, Ribaud P, De La Camara R, Martino R, Ullmann AJ, Parkkali T, Locasciulli A, Yakouben K, et al. Randomized study of early versus late immunization

with pneumococcal conjugate vaccine after allogeneic stem cell transplantation. Clin Infect Dis. 2009;48:1392-1401.

20. Lindström V, Aittoniemi J, Salmenniemi U, Käyhty H, Huhtala H, Itälä-Remes M, Sinisalo M. Antibody persistence after pneumococcal conjugate vaccination in patients with chronic lymphocytic leukemia. Hum Vaccin Immunother. 2018;14:1471-1474.

21. Simell B, Lahdenkari M, Reunanen A, Käyhty H, Väkeväinen M. Effects of ageing and gender on naturally acquired antibodies to pneumococcal capsular polysaccharides and virulence-associated proteins. Clin Vaccine Immunol. 2008;15:1391-1397.

22. Jodar L, Butler J, Carlone G, Dagan R, Goldblatt D, Käyhty H, Klugman K, Plikaytis B, Siber G, Kohberger R, et al. Chang I, Cherian T. Serological criteria for evaluation and licensure of new pneumococcal conjugate vaccine formulations for use in infants. Vaccine 2003;21:3265-3272.

23. Farmaki PF, Chini MC, Mangafas NM, Tzanoudaki MT, Piperi CP, Lazanas MZ, Spoulou VS. Immunogenicity and immunological memory induced by the 13-valent pneumococcal conjugate followed by the 23-valent polysaccharide vaccine in HIV-infected adults. J Infect Dis. 2018;218:26-34.

24. Papadatou I, Spoulou V. Pneumococcal vaccination in high-risk individuals: Are we doing it right? Clin Vaccine Immunol. 2016;23:388-395.

25. Kobayashi M, Bennett NM, Gierke R, Almendares O, Moore MR, Whitney CG, Pilishvili T. Intervals between PCV13 and PPSV23 vaccines: Recommendations of the advisory committee on immunization practices (ACIP). MMWR Morb Mortal Wkly Rep. 2015;34:944-947.

26. Orange JS, Ballow M, Stiehm ER, Ballas ZK, Chinen J, De La Morena M, Kumararatne D, Harville TO, Hesterberg P, Koleilat M, et al. Use and interpretation of diagnostic vaccination in primary immunodeficiency: A working group report of the basic and clinical immunology interest section of the american academy of allergy, asthma & immunology. J Allergy Clin Immunol. 2012;130:S1-24.

Table 1. Clinical and laboratory characteristics of the patients with CLL.

Character	Patients with CLL (n=20)
Sex M/F	11/9
Age (years)	63 (47-86)
Binet A/B/C	12/2/6
Past CLL therapy	7 (35%)
Lymphocyte count ($x10^9$/l)	21.9 (0.9-140.0)
Platelet count ($x10^9$/l)	141 (38-372)
Hemoglobin (g/l)	135 (81-153)
Neutrophil count ($x10^9$/l	4.0 (0.6-12.1)
IgG (g/l)	7.4 (3.2-12.5)

The values are expressed as medians and ranges.

Table 2. Pre- and post-vaccination antibody concentrations to seven serotypes included in the 7-valent pneumococcal conjugate vaccine (PCV7, bold) and two serotypes included in the 23-valent pneumococcal polysaccharide vaccine (PPV23) in patients with CLL and in controls.

Serotype	Pre-vaccination antibody concentration GMC (μg/ml) (quartiles)		Post-vaccination antibody concentration GMC (μg/ml) (quartiles)		p-value[a]
	CLL (n=20)	Control (n=8)	CLL (n=20)	Control (n=8)	
4	**0.12 (0.02-0.40)**	**0.34 (0.13-0.90)**	**0.13 (0.02-0.67)**	**1.96 (1.36-2.82)**	**<0.001**
5	0.16 (0.08-0.44)	0.15 (0.08-0.25)	0.22 (0.08-0.64)	2.20 (0.53-5.57)	0.002
6B	**0.46 (0.11-1.69)**	**0.30 (0.06-1.24**	**0.56 (0.14-3.39)**	**2.28 (0.75-9.96)**	**0.063**
7F	0.38 (0.07-1.27)	0.56 (0.31-1.23)	0.55 (0.07-1.83)	6.74 (2.97-13.8)	<0.001
9V	**0.51 (0.23-2.33)**	**0.91 (0.17-5.06)**	**0.62 (0.32-2.29)**	**3.04 (0.58-12.1)**	**0.033**
14	**0.77 (0.20-4.14)**	**1.56 (0.45-5.83)**	**0.96 (0.17-6.20)**	**6.65 (3.00-24.0)**	**0.021**
18C	**0.93 (0.26-3.06)**	**1.55 (1.00-2.47)**	**1.06 (0.27-4.07)**	**6.81 (3.82-15.7)**	**0.010**
19F	**0.97 (0.30-2.88)**	**0.83 (0.31-1.56)**	**1.29 (0.33-4.20)**	**4.18 (1.88-6.05)**	**0.055**
23F	**0.53 (0.14-1.56)**	**0.82 (0.25-2.23**	**0.67 (0.13-4.41)**	**2.29 (2.07-3.32)**	**0.150**

[a]Between post-vaccination concentrations in patients with CLL and controlS

[a]Independent-samples Mann-Whitney U-test (statistical significance if $p \leq .05$)

Table 3. Significant response rates and post-vaccination antibody concentrations suggestive of protection (≥0.35 μg/ml) to seven serotypes included in PCV7 (bold) and two serotypes included in PPV23 in patients with CLL and controls.

	Response rate[a]			Post-vaccination concentration ≥0.35 μg/ml	
	CLL	Controls		CLL	Controls
Serotype	n=20 (%)	n=8 (%)	p-value[b]	n=20 (%)	n=8 (%
4	**2 (10)**	**7 (88)**	**<0.001**	**7 (35)**	**8 (100)**
5	4 (20)	8 (100)	<0.001	6 (30)	8 (100)
6B	**2 (10)**	**6 (75)**	**0.002**	**10 (50)**	**7 (88)**
7F	3 (15)	6 (75)	<0.001	14 (70)	8 (100)
9V	**3 (15)**	**7 (88)**	**0.001**	**13 (65)**	**8 (100)**
14	**2 (10)**	**7 (88)**	**<0.001**	**12 (60)**	**8 (100)**
18C	**2 (10)**	**6 (75)**	**0.002**	**15 (75)**	**8 (100)**
19F	**3 (15)**	**7 (88)**	**0.001**	**15 (75)**	**8 (100)**
23F	**2 (10)**	**4 (50)**	**0.038**	**13 (65)**	**8 (100)**

[a]Defined as an at least 2-fold increase and a post-vaccination concentration of at least 0.35 μg/ml
[b]Fisher´s exact test