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Temporary reactive response of axillary lymph nodes to COVID-19 vaccination on ^{18}F-rhPSMA-7.3 PET/CT in patients with prostate cancer

Short title: Post COVID-19 vaccine on PSMA PET/CT

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ABSTRACT

Vaccine-associated lymphadenopathy (VAL) is a common finding on ^{18}F-FDG PET/CT examinations following Coronavirus disease 2019 (COVID-19) vaccination. However, data regarding VAL on ^{18}F-rhPSMA-7.3-ligand PET are currently lacking. This study assesses its prevalence, temporal response to vaccination and characteristics of VAL. **Methods:** 233 consecutive vaccinated and 41 unvaccinated patients with confirmed prostate cancer who underwent ^{18}F-rhPSMA-7.3 PET/CT were retrospectively analyzed. Size and uptake of the axillary lymph nodes were measured. Ratios of SUVmax of ipsilateral to contralateral axillary lymph node (SUVratio) were determined. The characteristics of SUVratio in respect to the duration of PSMA avidity in axillary lymph node after COVID-19 vaccination was analyzed. **Results:** The prevalence of VAL on ^{18}F-rhPSMA-7.3 PET was 45%. Up to a period of 8 weeks following last COVID-19 vaccination SUVratio was positive (2.05 ± 0.17). Thereafter, SUVratio dropped significantly (1.35 ± 0.09) and approached the value of unvaccinated group (1.1 ± 0.2). SUVratio of metastatic axillary lymph nodes was very high (>11) and can be easily detected visually or semi quantitatively. In 3 patients we observed suspected development and consecutively confirmed involving metastasis of axillary lymph node with SUVratio between 4.0 to 6.6. Correlation between SUVratio and lymph node size ($r=0.93$, $p<0.0001$) and lymph node size and duration following vaccine ($r=-0.88$, $p<0.0008$) was found. **Conclusion:** Increased uptake of the PSMA-ligand ^{18}F-rhPSMA-7.3 by axillary lymph nodes is common after COVID-19 vaccination and can persist for 8 weeks. This finding should be considered in the interpretation of ^{18}F-rhPSMA-7.3 PET/CT examinations.

KEY WORDS

COVID-19 vaccine, PSMA-ligand PET/CT, axillary lymphadenopathy, rhPSMA

ABBREVIATIONS LIST

COVID-19	Coronavirus disease 2019
CT	Computed tomography
PET	Positron emission tomography
PSMA	Prostate-specific membrane antigen
SUVmax	Maximal standardized uptake value
SUVratio	Ratio of SUVmax ipsilateral / contralateral lymph node
VAL	Vaccine-associated lymphadenopathy

INTRODUCTION

In times of global pandemic of Coronavirus disease 2019 (COVID-19) infections and rapidly increasing vaccinated population, Vaccine-Associated Lymphadenopathy (VAL) in axillary or supraclavicular lymph nodes ipsilateral to the vaccination site on ^{18}F-FDG examinations was increasingly observed (*1-6*). Ipsilateral axillary lymphadenopathy following intramuscular vaccine has been observed with seasonal and H1N1 influenza and human papilloma virus vaccines (*7-10*). These findings can impede interpretation of PET imaging, which poses an additional challenge for the workflow in nuclear medicine departments during the pandemic (*11,12*). The recognition of false-positive results is crucial to avoid unnecessary surgical re-exploration or medical therapies. This has also been recognized by multidisciplinary recommendations of the scientific expert panel (*13*). However, to our best knowledge, there are only very limited data to which extent COVID-19 vaccinations induced increased uptake of PSMA ligands by regional lymph nodes (*5,14*). Given the recent approval of two PSMA imaging agents and the high incidence and prevalence of prostate cancer, the most common malignancy in men, it is very likely that many men will undergo PSMA PET imaging after a recent COVID-19 vaccination.

This study aimed to retrospectively assess the rate of VAL in PSMA-ligand PET/CT and to investigate the characteristics and compare it with those of unvaccinated patients.

MATERIALS AND METHODS

Patient Population

Between June and July 2021, a total of 265 consecutive patients with histologically confirmed intermediate/high risk prostate cancer who underwent ^{18}F-rhPSMA-7.3 PET/CT, either for staging, restaging or planning of PSMA targeted radionuclide therapy were included in this retrospective study. In addition, the examinations of 9 additional advanced prostate cancer

patients with known axillary lymph node metastases were analyzed in order to compare the features of axillary lymph node metastases with vaccination related changes. In 3 of the 265 patients, clinical follow-up provided evidence for axillary lymph node metastases. These patients were subsequently excluded and were assigned and added to the group with known axillary lymph node metastases. All examinations were performed for clinical purposes as described by national and European guidelines (*11*,*15*).

In all patients, the date and the side of COVID-19 vaccination were recorded in the clinical notes. Informed written consent was obtained from all patients. The retrospective study has been approved by the institutional review board (Approval 719/21 S-NP).

Synthesis of ^{18}F-rhPSMA-7.3 and Administered Activity

^{18}F-rhPSMA-7.3 was synthesized as described previously (*16*) under a license by the local authorities (Regierung von Oberbayern). Body weight adapted activity with a median of 249 MBq of ^{18}F-rhPSMA-7.3 (mean 258±46, range 174–400 MBq) was administered as an intravenous bolus with a median of 75 minutes (mean 74±11 range 60–118) before examination.

PET/CT Acquisition

All PET/CT examinations were performed on either a Biograph-Vision 600 or Biograph mCT scanner (Siemens Healthineers, Erlangen, Germany). All patients received diluted oral contrast (300 mg of ioxitalamate [Telebrix; Guerbet]) and 20 mg furosemide. A diagnostic CT imaging was performed in the portal venous phase 80 seconds after intravenous injection of contrast agent [Imeron 300] (1.5 ml/kg body weight, max. 120 ml) followed by the PET imaging in flow-mode. All PET examinations were acquired in 3D mode with an acquisition time of 1.1 mm/second. Emission data were corrected for randoms, dead time, scatter, and attenuation and

were reconstructed iteratively by an ordered-subsets expectation maximization algorithm (four iterations, eight subsets) including time-of-flight information and point spread function correction followed by a post-reconstruction smoothing Gaussian filter (5 mm full width at one-half maximum). PET/CT images were reviewed by two dual-board certified radiologist and nuclear medicine (CL and ME).

Image Analysis

Axillary lymph node radiotracer maximal standardized uptake values (SUVmax), normalized for body weight, were measured by placing a region of interest at the axillary lymph node in the ipsilateral and contralateral side of the axillae. The corresponding CT images serve as orientation for the exact localization of the region of interest. Maximal diameter of the lymph node on CT was measured. The evaluating physician (SN) was blinded for the site and time of the vaccination.

As SUVmax values depend on variable technical aspects, such as the use of different scanner, variable starting acquisition time after tracer injection, and on patients' conditions, such as clinical and oncological status, the ratio between the SUVmax of the lymph node in the ipsilateral and contralateral reference sites (SUVratio) was calculated. Axillary lymph node uptake was defined as positive in case the ratio $\geq$ 1.5 as described previously by Thomassen et al (*10*) for ^{18}F-FDG and Eifer et al (*5*). For unvaccinated patients, SUVratio was calculated as a ratio between SUVmax of left to that of the right axillary lymph node.

Statistical Analysis

Continuous variables were analyzed by descriptive statistics including arithmetic mean, standard deviation, median and range, whereas categorical variables were investigated by

frequencies. Data of SUVratio were grouped according to the duration of the final vaccination in weeks and were plotted as a diagram. Kruskal-Wallis-Test was used to compare SUVratio among patient groups with different time intervals between vaccine and PET examination. Wilcoxon rank-sum test was used to compare SUVratio between patient groups with and without vaccine, as well as the pairwise comparison between patient groups in two different time intervals between vaccine and PET examination. The correlation between SUVratio and lymph node size as well as between lymph node size and elapsed time since the latest vaccination was analysed by Spearman rank correlation. For all statistical analyses, statistical software SPSS (ver. 25, IBM) was used. Significance level α was set at 0.05 with $p<\alpha$.

RESULTS

233/274 patients (85%) were vaccinated against COVID-19, either with mRNA or attenuated viral vectors based approved vaccines, while 41 patients (15%) were not vaccinated. 262 of 274 patients revealed no axillary lymph node metastasis and the remaining 12 patients revealed axillary lymph node metastasis as confirmed by clinical data, lymph node exploration, and/or follow-up investigation.

Of the 233 vaccinated patients, 105 (45%) were noted to have increased PSMA-ligand uptake in ipsilateral axillary lymph node compared to the contralateral site with positive SUVratio of $\geq$ 1.5.

The time course of PSMA-ligand uptake in axillary lymph nodes after COVID-19 vaccination is shown in figure 1. The temporal changes in lymph node SUVratio and size are listed in Table 1. Patients initially grouped in week 1-8 were regrouped in group A, respectively, patients initially grouped in week 12-28 were regrouped in group B. The comparison of SUVratio among the group in A and in B revealed no significant differences, whilst between A and B a significant

decrease of SUVratio in B was registered ($p < 0.02$) and approached almost the level of unvaccinated patients. After 8 weeks, the average lymph node size also dropped significantly ($p < 0.01$). The SUVratio and lymph node size in unvaccinated patients was 1.1 ± 0.2 (range 0.6 – 1.5) and 11 ± 6 mm (range 6 – 18 mm).

SUVratio and lymph node size were significantly correlated ($r = 0.93$ $p<0.0001$, Fig. 2), and in the group of vaccinated patients there was a significant trend for the size of axillary lymph node to decrease over time following vaccination ($r = -0.88$ $p < 0.0008$, Fig. 3).

Confirmed axillary lymph node metastases had higher PSMA SUVratio of 14.92 ± 5.52 and were larger, with average size of 34 ± 12 mm, than non-metastatic nodes ($p < 0.001$ and $p < 0.001$, respectively). In patients without axillary lymph node metastasis the SUVratio and lymph node size after COVID-19 vaccination was 1.74 ± 0.87 (range 0.3 – 4.7) and 22 ± 9 mm (range 8 – 33 mm). Figure 4 represents an example of images of VAL in a patient without axillary lymph node metastasis and in another patient with axillary lymph node metastasis.

The subsequently confirmed metastatic axillary lymph nodes (n=3) exhibited focal PSMA ligand uptake within groups of lymph nodes of variable size (8-33 mm). The SUVratio was 5.1 ± 1.35 (range 4.0-6.6). These patients also had additional PSMA expressing lesions in ribs and thoracic vertebrae.

DISCUSSION

Increased ipsilateral axillary uptake of PSMA ligand is common and occurred in 45% of prostate cancer imaged at varying time intervals after COVID vaccination. This should be considered a source of false positive findings in PSMA PET/CT. The increased PSMA ligand bindings appears to resolve 8 weeks after vaccination. A recently published study where VAL was assessed in a small subgroup of 31 patients with prostate cancer and PSMA-ligand PET/CT

showed similar findings of PSMA-avid lymph nodes after vaccination (*14*). The prevalence of VAL in our population was lower compared to the previous study, presumably because we included a larger population with patients with a considerably longer period of time since vaccination. Eight weeks following the vaccination, axillary lymph node uptake was no longer significantly different from unvaccinated patients. The frequency of positive axillary lymph nodes on PSMA-ligand PET/CT examinations in our series is comparable to recently published data for ^{18}F-FDG PET/CT examinations following COVID-19 vaccinations (*1-5,13,17*). It is well established that activated lymphatic cells show increased metabolic activity and consequently accumulate more ^{18}F-FDG. In contrast, the reasons for the increased uptake of the ^{18}F-rhPSMA-7.3 are less clear. Contrary to its name, PSMA-expression is not specific to prostate and PSMA is expressed in many other organs, including lymph nodes (*18,19*). We believe that in addition to the physiological PSMA expression, COVID-19 vaccination-induced cellular and humoral immune responses in lymph nodes could augment the pre-existing PSMA expression and thus enhance the avidity of PSMA ligands in non-metastatic lymph nodes in patients with prostate cancer. Future research is necessary to determine if uptake of small molecule PSMA-ligands by inflammatory lesions is due to off-target binding (e.g. to peptidases different from PSMA) or due to PSMA expression that has so far remained undetected by immunohistochemistry.

Furthermore, our results may impact in clinical practice in sense of precautions in scheduling, preparation and interpretation of PSMA PET examination. There was significant overlap between PSMA ligand uptake in benign versus malignant axillary lymph nodes. Thus, intensity of tracer uptake cannot reliably distinguish between metastatic and inflammatory nodes following COVID-19 vaccination. Nevertheless, axillary lymph node uptake following COVID-19 vaccination alone should not be used as a criterion for tumor progression. Similar precautions than

recently published for ^{18}F-FDG PET/CT examinations are probably reasonable for the interpretations of PSMA-ligand PET/CT examinations after COVID-19 vaccinations (*13*).

The negative correlation between lymph node size and duration following vaccination indicates that statistically the average lymph node size tends to be smaller with increasing duration following vaccination. Our data is in concordance with the results reported previously on the basis of axillary-ultrasound imaging (*20*). The positive correlation between SUVratio and lymph node size indicates that statistically SUVratio tends to increase with increasing size of lymph nodes.

Limitations

In this study, only patients vaccinated with FDA and EMA (European Medicines Agency) approved COVID-19 vaccines (BioNTech-Pfizer, Moderna, Astra Zeneca, Johnson & Johnson) were included. Despite known differences in the immunological response of the vaccines, separate analysis among those vaccines was not performed because this information had not been consistently reported in the clinical notes.

Since local clinical symptoms vary between different COVID-19 vaccines, future research is required if there are also differences between the vaccines with respect to PSMA-ligand uptake.

We only studied patients imaged with ^{18}F-rhPSMA-7.3, which is currently in late-stage clinical trials for prostate cancer imaging. The impact of COVID-19 vaccination on other PSMA-ligands remains to be studied.

CONCLUSIONS

PSMA avid lymphadenopathy is common after COVID-19 vaccination. The time required after COVID-19 vaccination to allow for resolution of PSMA uptake of reactive axillary lymph

node was 8 weeks. During this period care must be taken to avoid false positive findings on PSMA-ligand PET/CT examinations.

DISCLOSURE

Matthias Eiber is named as an inventor on a patent application for ^{18}F-rhPSMA-7.3, reports research support from Blue Earth Diagnostics Ltd., and prior consulting activities for Blue Earth Diagnostic Ltd., Novartis, Telix, Progenics, Bayer, Point Biopharma and Janssen. No other potential conflict of interest relevant to this article was reported.

Wolfgang Weber has indicated that he is on advisory boards and receives compensation from Bayer, Blue Earth Diagnostics, Endocyte, Reflexion, Rayzebio, Vida Ventures, ITM and Pentixapharm. He has received research support from Siemens, BMS, Ipsen, Imaginab and Piramal. No other potential conflicts of interest were reported.

Susan Notohamiprodjo and Christian Lohrmann have nothing to disclose.

KEYPOINTS

Question: What is the prevalence of vaccine-associated lymphadenopathy (VAL) in PSMA-PET? How long does VAL persist in PSMA-PET?

Pertinent Findings: The prevalence of VAL is 45% (in 105 from 233 vaccinated patients) and thus common. Temporary occurrence of VAL persists until 8 weeks after last vaccination.

Implication for Patient Care: During 8 weeks following COVID-19 vaccination care must be taken to avoid false positive findings on PSMA PET/CT examinations.

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Graphical Abstract

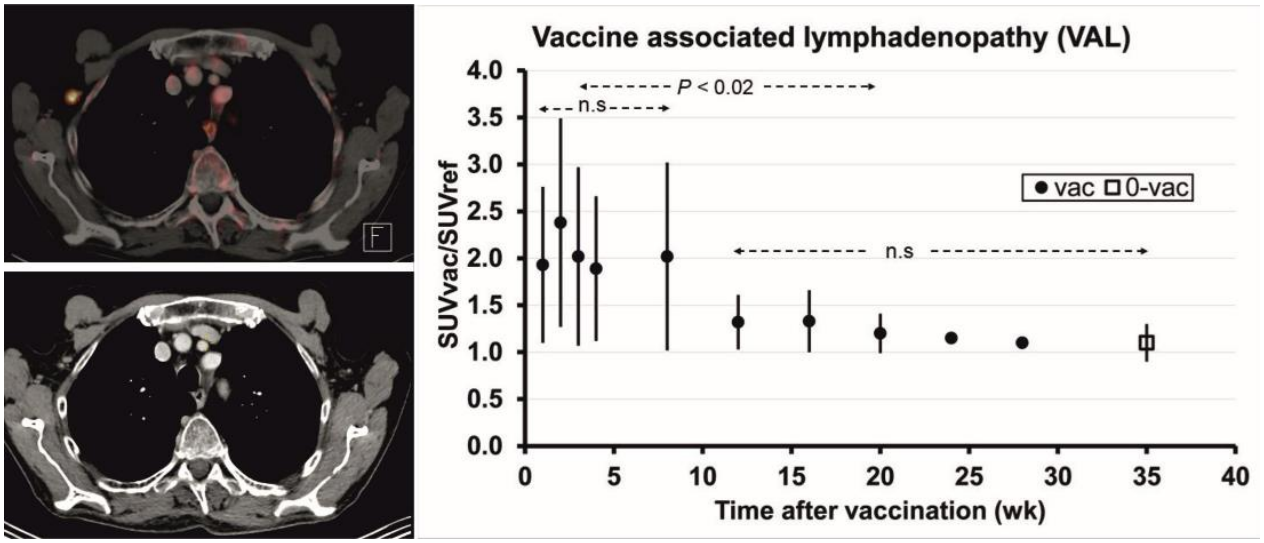
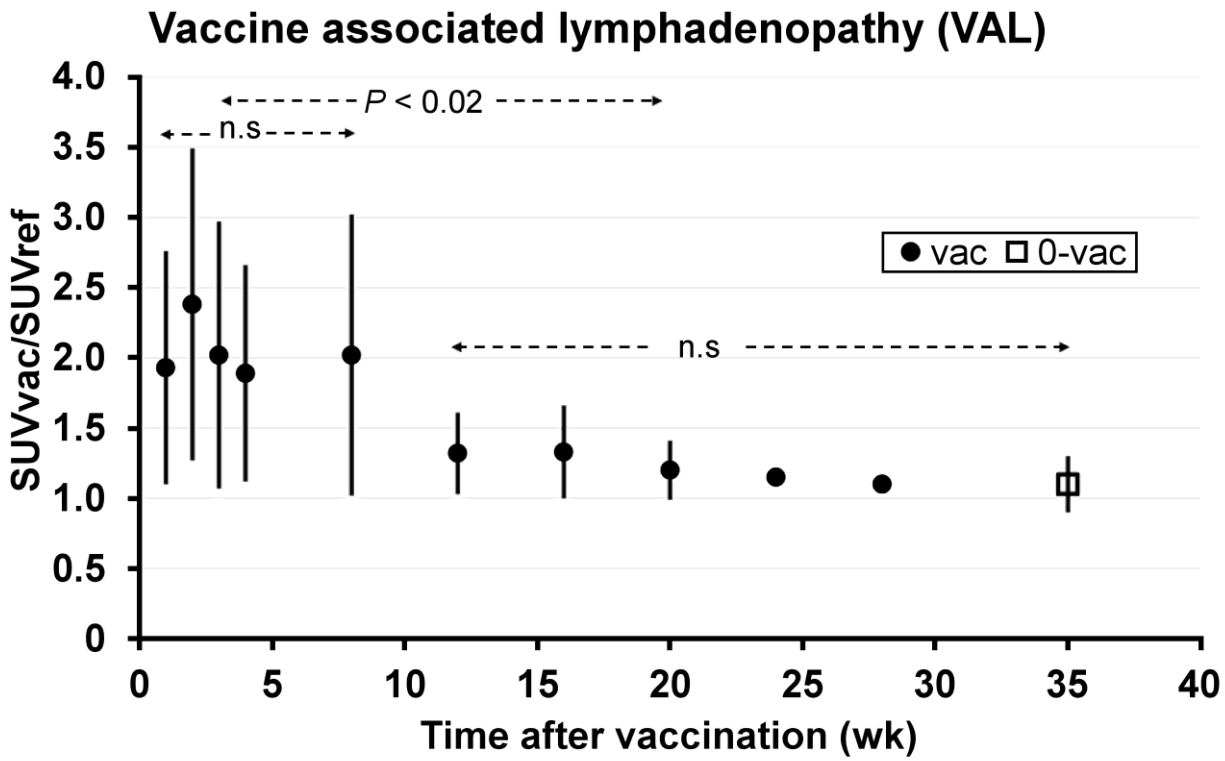
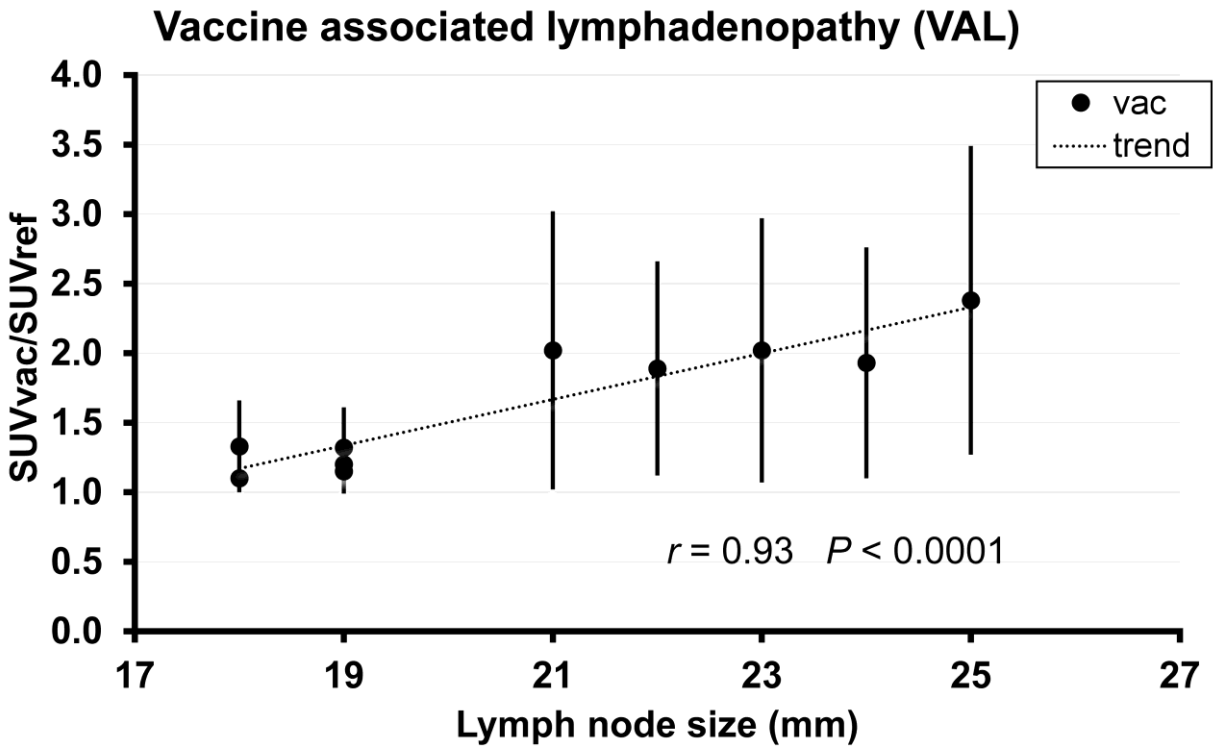


Fig. 1 Temporal response of SUVratio following COVID-19 vaccination on PSMA PET/CT. Error bars indicate standard deviation.



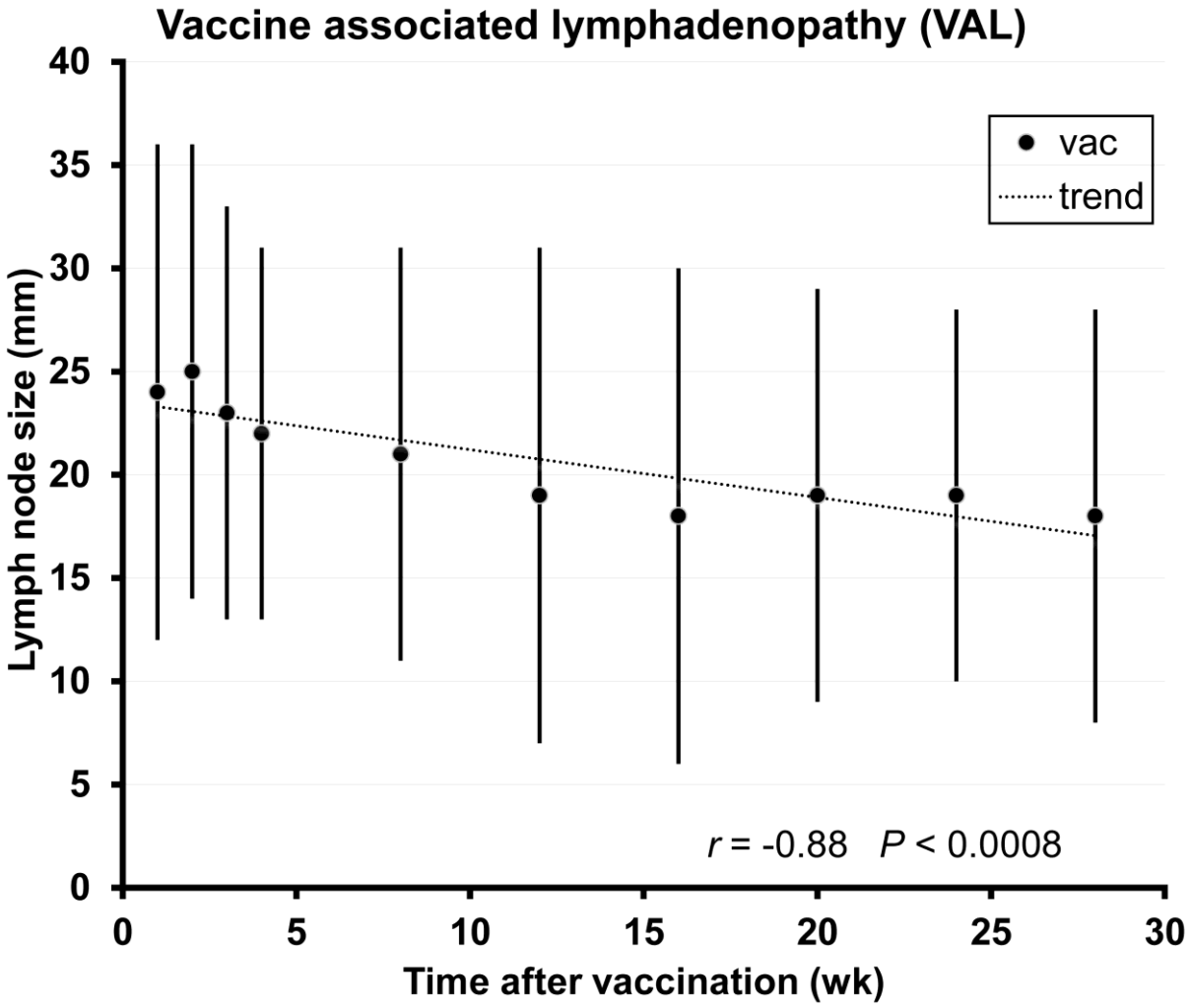
vac = vaccinated, 0-vac = unvaccinated, n.s = statistically not significant

Fig. 2 Relation between SUVratio and lymph node size on PSMA PET/CT. Error bars indicate standard deviation.



vac = vaccinated. Error bars indicate standard deviation

Fig. 3 Relation between lymph node size and duration following vaccination. Error bars indicate standard deviation.



vac = vaccinated. Error bars indicate standard deviation

Fig. 4 Representative PSMA PET/CT images of a patient with VAL (A) and of another patient with axillary lymph node metastasis (B).

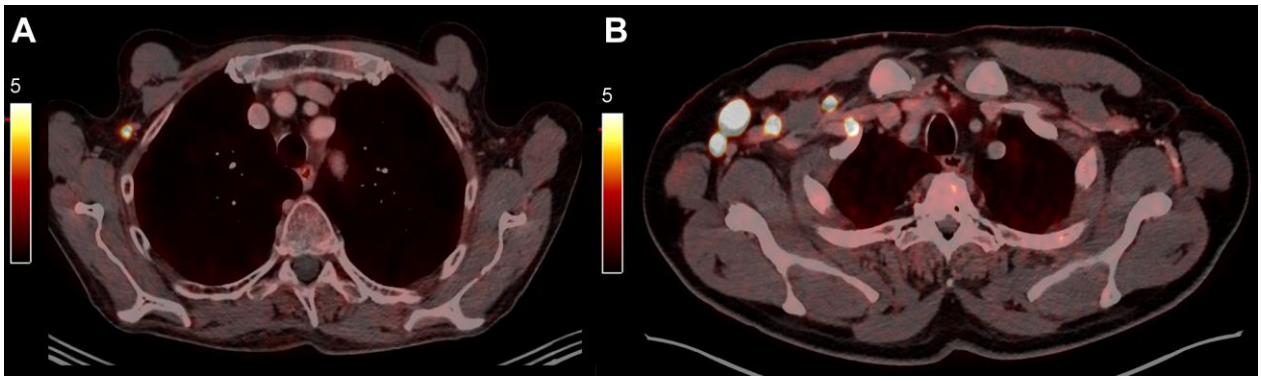


TABLE 1

Temporal changes of average mean SUVratio and average mean size of axillary lymph nodes of patients without axillary lymph node metastasis and of non-vaccinated patients.

Group	**A**	**B**	**C**
Time of vaccination	between week 1 – week 8	between week 8 – week 28	no-vac
Average mean SUVratio	2.05	1.35	1.10
SD (SUVratio)	$\pm$ 0.17	$\pm$ 0.09	$\pm$ 0.20
p-value comparison SUVratio between A and B → $p < 0.02$			
Average mean size	23 mm	19 mm	11 mm
SD (size)	$\pm$ 10 mm	$\pm$ 11 mm	$\pm$ 6 mm
p-value comparison lymph node size between A and B → $p < 0.01$			
*Patients initially grouped in week 1, week 2, week 3, week 4 and week 8 were regrouped in **A*** *Patients initially grouped in week 12, week 16, week 20, week 24 and week 28 were regrouped in **B*** *SD = standard deviation*			