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Absolute lymphocyte count after COVID-19 vaccination is associated with vaccine-induced hypermetabolic lymph nodes on ^{18}F-FDG PET/CT: a focus in breast cancer care.

SHORT RUNNING TITLE
Vaccine-induced lymph nodes on FDG-PET

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ABSTRACT

Rationale: We aimed to predict the presence of vaccine-induced hypermetabolic lymph nodes (v-HLN) on ^{18}F-FDG PET/CT after Coronavirus disease 2019 (COVID-19) vaccination and determine their association with lymphocyte counts.

Methods: In this retrospective single-center study, we included consecutive patients who underwent ^{18}F-FDG PET/CT imaging after mRNA- or viral vector-based COVID-19 vaccination between early March and late April 2021. Demographic, clinical parameters and absolute lymphocyte count (ALC) were collected and their association with the presence of v-HLN in the draining territory was studied by logistic regression.

Results: Two hundred and sixty patients were eligible, including 209 (80%) women and 145 (56%) with breast cancer. The median age was 50 years (range, 23-96). Two hundred thirty-three patients (90%) received the mRNA vaccine. Ninety (35%) patients had v-HLN with a median SUVmax of 3.7 [range, 2.0-26.3] and 74 (44%) displayed lymphopenia with a median ALC of 1.4 G/L [range, 0.3-18.3]. Age ≤ 50 years (odds ratio [OR] 2.2, 95%CI 1.0-4.5), the absence of lymphopenia (OR 2.2, 95%CI 1.1-4.3) and the delay from the last vaccine injection to the date of ^{18}F-FDG PET/CT, if < 30 days (OR 2.6, 95%CI 1.3-5.6), were independent factors for v-HLN in multivariate analysis. In breast cancer patients, the absence of lymphopenia was the only independent factor significantly associated with v-HLN (OR 2.9, 95%CI 1.2-7.4).

Conclusions: Patients with normal values of ALC after COVID-19 vaccine were more likely to have v-HLN on ^{18}F-FDG PET/CT, which might both be associated to a stronger immune response to vaccination.

KEYWORDS

^{18}F-FDG PET/CT; COVID-19 vaccination; absolute lymphocyte count; hypermetabolic lymph nodes; immune response.

INTRODUCTION

A billion doses have been administered worldwide using currently authorized and recommended Coronavirus disease 2019 (COVID-19) mRNA (*1*,*2*) or viral vector vaccines (*3*). Such vaccination has been shown to promote immunity against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) by inducing strong T and memory B cell responses (*4*,*5*).

Since the generation of an immune response increases glucose metabolism in lymphoid organs, which are critical modulators of T- and B-cell immunity (*6*,*7*), ^{18}F-FDG PET/CT could be used as a potent tool to assess immune response after vaccinations against several infections, including SARS-CoV-2 (*8*), influenza virus (*9*), human papillomavirus (*10*) but also cancer (*11*). Recently, several findings on ^{18}F-FDG PET/CT have been reported in patients vaccinated against COVID-19, most likely related to immune activation in lymphoid organs as well as hypermetabolic lymph node(s) (HLN) in the drainage territory (*12–19*) or an increased spleen glucose metabolism (*20–22*).

In patients who received mRNA vaccines for COVID-19, the prevalence of vaccine-induced HLN (v-HLN) on ^{18}F-FDG PET/CT was approximately 45% (*12*,*14*,*23*). Indeed, v-HLN were more commonly observed in young and immunocompetent patients (*14*). Additionally, the delay between the last vaccine dose and the number of vaccine doses were also significantly associated with the presence of v-HLN (*14*). Furthermore, v-HLN appeared to correlate with an effective humoral response induced by the mRNA vaccination (*13*). However, it remains unclear whether similar results would be obtained following the administration of another type of vaccine (e.g. viral vectors).

As well as for lymphoma, breast cancer (BC) patients are considerably more susceptible to be affected by v-HLN than other types of cancer, as stated by Cohen et al. (*12*). Among BC patients, v-HLN in the axillary area and beyond can mimic tumor lesions and lead to confounding imaging results (*16*). Recent publications emphasized the importance of documenting vaccination history at the time of scanning to avoid false-positive results (*21*,*24*) with all the attendant negative consequences: unnecessary biopsy/cytology or lymphadenectomy for early-stage BC or unjustified changes in systemic treatment for advanced-stage BC.

In the present study, we specifically aimed at predicting the presence of vaccine-induced

lymph nodes (v-HLN) on ^{18}F-FDG PET/CT after COVID-19 vaccination and investigating their relationships with lymphocyte counts, with a special focus on a sub-group of BC patients.

MATERIAL AND METHODS

Patients

We conducted a retrospective review of 702 consecutive patients who underwent ^{18}F-FDG PET/CT imaging at Institut Curie Hospital, Saint-Cloud, France (the flow chart is provided in figure 1). Four hundred forty-two patients were excluded based on the following criteria: (i) non-vaccinated patients (n=437); (ii) patients who expressed their opposition to participate in medical research (n=5). This retrospective data collection complied with our Institutional Review Board (DATA210128), with a waiver of informed consent ("rule of non-opposition") and the study was conducted according to the Declaration of Helsinki.

Clinicobiological data

Clinical data consisted of the patient's age, gender, disease (cancer type if applicable), current specific treatment (chemotherapy, endocrine therapy, immunotherapy with immune checkpoint inhibitors/ICIs, targeted therapy). All patients were asked to state the date of COVID-19 vaccination, type/brand of vaccine (mRNA or viral vector) and site in which the vaccine was injected on the first and second doses (if applicable).

We considered a sub-group of patients who theoretically had a weakened immune system, which we called "immunosuppressed", caused by the following treatments: chemotherapy within the last 3 months, rituximab-containing regimens or bone marrow transplantation within the last 6 months or current steroids therapy with >10 mg/day of prednisone-equivalent. Biological characteristics were obtained from peripheral blood samples before ^{18}F-FDG PET/CT imaging/after vaccination (at least one dose), including the absolute lymphocyte count (ALC). For ALC, we used the limit of each center (LLN—the lower limit of normal). The inclusion criteria for blood samples assessment before ^{18}F-FDG PET/CT imaging/after vaccination was 28 days.

^{18}F-FDG PET/CT scans

^{18}F-FDG PET/CT scans were performed in accordance with the applicable EANM procedure guidelines (*25*). Patients fasted for at least six hours before scanning to ensure a blood glucose

level < 10 mmol/L. Scanning was performed using a Philips Vereos PET/CT, combining the small LYSO with the SiPM block design (LightBurst digital detector). PET images were reconstructed with a fully 3D time-of-flight iterative reconstruction method (VPFX) (OSEM algorithm, matrix 288 x 288, 3 iterations, 5 subsets, post-filter 2mm). Images were converted to SUV units by normalization using the patient's body weight.

Measurement and interpretation of ^{18}F-FDG PET/CT parameters

Four certified nuclear medicine physicians (RDS, CR, ND and LC) analyzed ^{18}F-FDG PET/CT. Measures of mean or maximum standardized uptake values (SUVmean, SUVmax) were obtained from HLN detected in the drainage territory using the PET tumor segmentation tool in Philips IntelliSpace Portal 9.0. Readers were blinded and did not know the patient's characteristics.

Draining lymph node(s) uptake values were measured on the PET images, assisted by CT data for the anatomical location. As previously published by Thomassen et al. (*9*), we also recorded contralateral lymph nodes uptake values, which were used for reference. Similarly, HLN was defined as having a ratio ≥ 1.5 between SUVmax in the ipsilateral and contralateral reference sites (*9*,*14*). LN SUVmax was defined as the highest SUVmax among all HLN detected in the drainage territory (axillary and/or supra-clavicular in case of vaccination in the deltoid, inguinal in case of vaccination in the thigh or buttock). The size of the most HLN was recorded using short-axis diameter measurement on CT images. The categorization of vaccine-induced HLN (v-HLN) was obtained using the clinical background: type, stage and site of disease, histological findings (biopsy or cytology) and other available imaging (enhanced MRI, CT-scans or previous ^{18}F-FDG PET/CT exams). Where there is any uncertainty about its HLN's etiology (disease-related or vaccine-induced), we have categorized the patients into an "indeterminate" HLN group (i-HLN).

Statistical analysis

Continuous and categorical variables were reported as median with range (minimum and maximum) and as frequency and percentage, respectively. Factors associated with v-HLN were tested by logistic regression analysis using a stepwise Aikaike Information Criteria (AIC) method

for variable selection (*26*). All reported p values are two-sided and p values less than 5% were considered to be significant. Analyses were performed with R software (version 4.0.2) (*27*).

RESULTS

Whole cohort

Patient's characteristics

Table 1 summarizes the detailed demographic and clinico-biological characteristics and PET imaging parameters of the 260 patients. The median age was 50 years (range, 23-96) and 80% were women. Two hundred thirty-three patients (90%) received the mRNA vaccine, including one hundred ten (42%) who had the two doses. The median time between the last vaccination dose and ^{18}F-FDG PET/CT scan was 14 days (range, 1-51) for patients that received only one vaccine dose and 23 days (range, 1-67) for patients that received a second vaccine dose. More than half of patients were referred for BC (56%) and 24% were considered as "immunosuppressed" (n=62). Blood sample analysis was available in 170 patients before PET/after vaccination. Among them, median ALC was 1.4 G/L [range, 0.3-18.3] and 74 (44%) displayed lymphopenia. Overall, ninety patients (35%) had v-HLN with a median SUVmax of 3.7 [range, 2.0-26.3].

Association between v-HLN on ^{18}F-FDG PET/CT and clinicobiological parameters

Age ≤ 50 years (odds ratio [OR] 2.2, 95%CI 1.0-4.5), the absence of lymphopenia (OR 2.2, 95%CI 1.1-4.3) and the delay from the last vaccine injection to the date of ^{18}F-FDG PET/CT, if < 30 days (OR 2.6, 95%CI 1.3-5.6), were statistically significant factors associated with v-HLN (figure 2) in univariate analysis (table 2). All these following parameters remained independent predictors of the v-HLN status in multivariate analysis. Interestingly, among patients displaying lymphopenia after vaccination, we evaluate the dynamic of ALC and found that low ALC existed before vaccination in the majority of patients (67%, n=36) suggesting that lymphopenia was not related to vaccination. The immunosuppression, the type of vaccine and the number of doses were not associated with v-HLN.

Breast cancer (BC) cohort

Patient's characteristics

Table 1 summarizes the detailed demographic and clinicobiological characteristics and PET imaging parameters of 145 BC patients. All patients were women. About one-third had an early-stage BC and the remaining two-third had an advanced-stage BC, mainly treated with endocrine therapy +/- targeted therapy or chemotherapy. Whole blood counts were available in 106 BC before PET/after vaccination. Among them, median ALC was 1.5 G/L [range, 0.4-5.0] and 41 (39%) displayed lymphopenia. Fifty-six patients (37%) had v-HLN with a median SUVmax of 3.7.

Association between v-HLN on ^{18}F-FDG PET/CT and clinicobiological parameters

Logistic regression analysis summarizing the association between v-HLN on ^{18}F-FDG PET/CT following COVID-19 vaccination and clinicobiological parameters is provided in table 3. The absence of lymphopenia was the only independent factor significantly associated with v-HLN (OR 2.9, 95%CI 1.2-7.4). The patients' age, the immune status, the type of vaccine, the number of doses or the delay from the last vaccine injection to the date of ^{18}F-FDG PET/CT were not significantly associated with v-HLN in this sub-group of BC patients.

Early-stage BC patients with vaccination ipsilateral to the tumor

We identified 7 BC patients who had axillary HLN ipsilateral to the recently vaccinated arm but also ipsilateral to the known tumor. Their demographic characteristics, vaccination information's, ^{18}F-FDG PET/CT parameters, ALC and histological data are reported in table 4. Six of them had been histologically documented by fine-needle aspiration cytology (FNAC, n=3) or sentinel lymph node biopsy (SLNB, n=3). One patient (14%) was not investigated and began neo-adjuvant endocrine therapy. The planned partial mastectomy with SLNB will help determine the cause of HLN afterwards. While all FNAC evidenced signs of malignancy with tumor cells (43%) (figure 3), all SLNB revealed benign reactive changes (43%) (figure 4).

DISCUSSION

We have shown that patients' age (≤ 50 years), ALC (>LLN) or the timing of last injection dose (< 30 days) significantly correlated with vaccine-induced hypermetabolic lymph node (v-HLN) on ^{18}F-FDG PET/CT following COVID-19 vaccination in a retrospective cohort of 260 patients. Moreover, among patients with BC, the ALC before ^{18}F-FDG PET/CT remained the

most strongly implicated factor associated with the v-HLN status. Indeed, BC patients with a normal value of ALC were more likely to have v-HLN on ^{18}F-FDG PET/CT.

Our results are consistent with previously published data, suggesting that v-HLN are significantly less common in elderly patients or who received their last vaccine injection a few days before ^{18}F-FDG PET/CT (*14*). In addition to providing information on the HLN status, which may help nuclear medicine physicians for images interpretation and oncologists for medical management, these findings also raise the question of whether the COVID-19 vaccine is triggering a more robust immune response in this population (≤ 50 years or ALC > LLN). In the specific setting of hematologic malignancies, Cohen et al demonstrated that the rate of v-HLN after mRNA vaccination was significantly higher in patients with positive serology than those with negative serology (*13*). This essential result could probably be the missing link between the presence of v-HLN and the vaccine effectiveness that would induce a strong immune response and therefore, robust immunity.

Another interesting aspect of our work is that ALC after vaccination and before ^{18}F-FDG PET/CT was an independent factor significantly associated with v-HLN in the whole cohort of 260 patients, which was further reinforced by our findings in the specific cohort of 145 BC patients. Such important results have been previously demonstrated after vaccination, especially again the SARS-CoV-2 (*28*,*29*) but also against other viruses (*30*). Indeed, Achiron et al. showed a correlation between the level of SARS-CoV-2 antibodies on serology and lymphocyte count at 1 month after the second dose in a cohort of 125 multiple sclerosis patients, which were fully vaccinated with BNT162b2-COVID-19 vaccine (*28*). These results have been confirmed in 427 patients with hematologic malignancies who also received two doses of with BNT162b2-COVID-19 vaccine, explored by serology (*29*). In this study, ALC correlated with higher seropositivity likelihood and antibody titers. This observation is not surprising given the pivotal role of lymphocytes in the immune response, specifically because these are instrumental in the formation of antibodies (*31*). All these parameters are closely related to the humoral immune response; however, as stated by the authors of the previously cited studies, the role of lymphocytes in in cell-mediated immune response following COVID-19 vaccination remains to be investigated.

Based on our findings, there is no evidence to support the conclusion that immunosuppression

leads to a lower incidence of v-HLN. While we could say that chemotherapy or rituximab-containing regimens are likely to block the serologic response to COVID-19 (*13*) or influenza A (H1N1) vaccinations (*32*,*33*), the relationship between immunodepression and reactive hypermetabolic LN in the drainage territory remains unclear regarding the literature. On the one hand, Thomassen et al. showed that immunosuppressive drugs given within 2 weeks from vaccination did not affect the axillary LN's uptake in 293 patients who had been vaccinated with at least one influenza vaccination in the deltoid region (*9*). On the other hand, Cohen et al. revealed that lymphoma patients treated during the year before COVID-19 vaccination with rituximab-containing regimens (9%) had significantly lower rates of v-HLN compared with all other lymphoma patients (41%), associated with a strong relationship between v-HLN and positive serologies (Spearman's correlation coefficient: rho = 0.64 in patients who received the two doses of mRNA vaccine) (*13*). These results are strengthened by Eifer's study, demonstrating a strong inverse association between v-HLN and immunosuppressive therapies (OR=0.37, 95% CI, 0.20-0.64; $p<0.01$) in a large cohort of 377 patients following mRNA-based COVID-19 vaccination (*14*). However, determinants correlated with the high glucose metabolism in the LN could be multiple, with, for example, age or lymphocyte count or timing of last injection dose, with a higher degree of association, which requires further analyses to explore the specific relationship between v-HLN, the immune status and the immune response to the COVID-19 vaccine.

In the specific case of early-stage BC patients, it is usually recommended to do the vaccination on the opposite arm relative to the breast cancer side. However, in rare cases, early-stage BC patients could have bilateral cancers or receive the vaccine injection in the arm ipsilateral to the known tumor, which might falsely influence the PET report. We thus studied patients with axillary HLN ipsilateral to the recently vaccinated arm but also ipsilateral to the known tumor, and we found signs of malignancy with tumor cells in half of the patients while the other half had benign reactive changes. The sample size (n=7) was too small to provide a statistical analysis or draw any conclusion. Unfortunately, blood sample analysis was not available for all these patients, so we could not determine if patients with benign reactive changes had significantly higher ALC than patients with signs of malignancy. Since the date of PET examination could hardly be postponed in cancer patients, predicting the nature of HLN on ^{18}F-FDG PET/CT has become an area of intensive investigation to avoid unnecessary biopsies or aggressive treatments.

As a result, the three parameters (age, timing of last injection dose and ALC) that we have identified in the current study may help to guide nuclear medicine physicians in interpreting ^{18}F-FDG PET/CT images and oncologists in choosing whether or not to perform a biopsy. Further research is required to validate such findings and identify clinical, biological and imaging factors associated with the nature of HLN (benign versus malignant) ipsilateral to the breast tumor in a larger cohort of patients with early-stage BC.

The strength of our study is the large sample size. The main limitation concerns the retrospective nature and single-center study. We did not include gender in our logistic regression analysis because of a sample-selection bias, explained by the predominance of women which accounts for 80 per cent. Indeed, this gender bias in favor of women is due to the nature of our center, which is a referral one for BC treatment. Moreover, only ten per cent of patients received the viral vector based-COVID-19 vaccine. Consequently, we can't really conclude with sufficient power on the specific vaccine-subtype effects on the ^{18}F-FDG PET/CT response to SARS-CoV-2 vaccination in the present study.

Studies deciphering the metabolic patterns on ^{18}F-FDG PET/CT after vaccination are needed since people might need to get vaccinated against the SARS-CoV-2 annually.
In any case, our work confirms the potential of ^{18}F-FDG PET/CT as a potent tool to assess immune response after COVID-19 vaccination, which can be explained by the fact that immune response increases glucose metabolism in lymphoid organs (e.g regional lymph nodes), which are critical modulators of immunity (*34*). Now proven to be more than 90% effective against SARS-CoV-2, the mRNA technology will probably modify the therapeutic armamentarium in patients with solid malignant tumors (*35*,*36*). However, it remains to be demonstrated that ^{18}F-FDG PET/CT can thus become a relevant imaging tool, which would assess healthy lymphoid tissues for in vivo quantification of the immune response after mRNA vaccination in its widest sense (*8*).

CONCLUSION

In this large cohort of 260 patients, we demonstrated that patients' ALC was a critical determinant of v-HLN on ^{18}F-FDG PET/CT following mRNA based- or viral vector based-COVID-19 vaccination, as well as patients' age and timing of last injection dose. In BC patients, normal values of ALC after vaccination and before ^{18}F-FDG PET/CT was the best indicator of

the v-HLN status. Both of these interrelated elements (age and ALC) could modulate the quality of the immune response after COVID-19 vaccination. Further prospective studies are warranted to investigate whether the metabolism of lymphoid organs on ^{18}F-FDG PET/CT is a crucial effector for the success of the immune response after COVID-19 vaccination.

DISCLOSURE

The authors declare that they have no conflict of interest.

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None.

KEY-POINTS

QUESTION: Could we use lymphocyte count lymphocyte for predicting the presence of vaccine-induced hypermetabolic lymph node(s) in the drainage territory on ^{18}F-FDG PET/CT following COVID-19 vaccination?

PERTINENT FINDINGS: This retrospective and monocentric study included 260 vaccinated patients who underwent ^{18}F-FDG PET/CT following mRNA based- or viral vector based-COVID-19 vaccination. Patients' absolute lymphocyte count (>lower limit of normal), along with patients' age (≤ 50 years) and the timing of last injection dose (< 30 days), significantly correlated with vaccine-induced hypermetabolic lymph node(s).

IMPLICATIONS FOR PATIENT CARE: Patients displaying normal count of lymphocytes after COVID-19 vaccination are more likely to present vaccine-induced hypermetabolic lymph node(s) on ^{18}F-FDG PET/CT and could subsequently have higher seropositivity likelihood and antibody titers.

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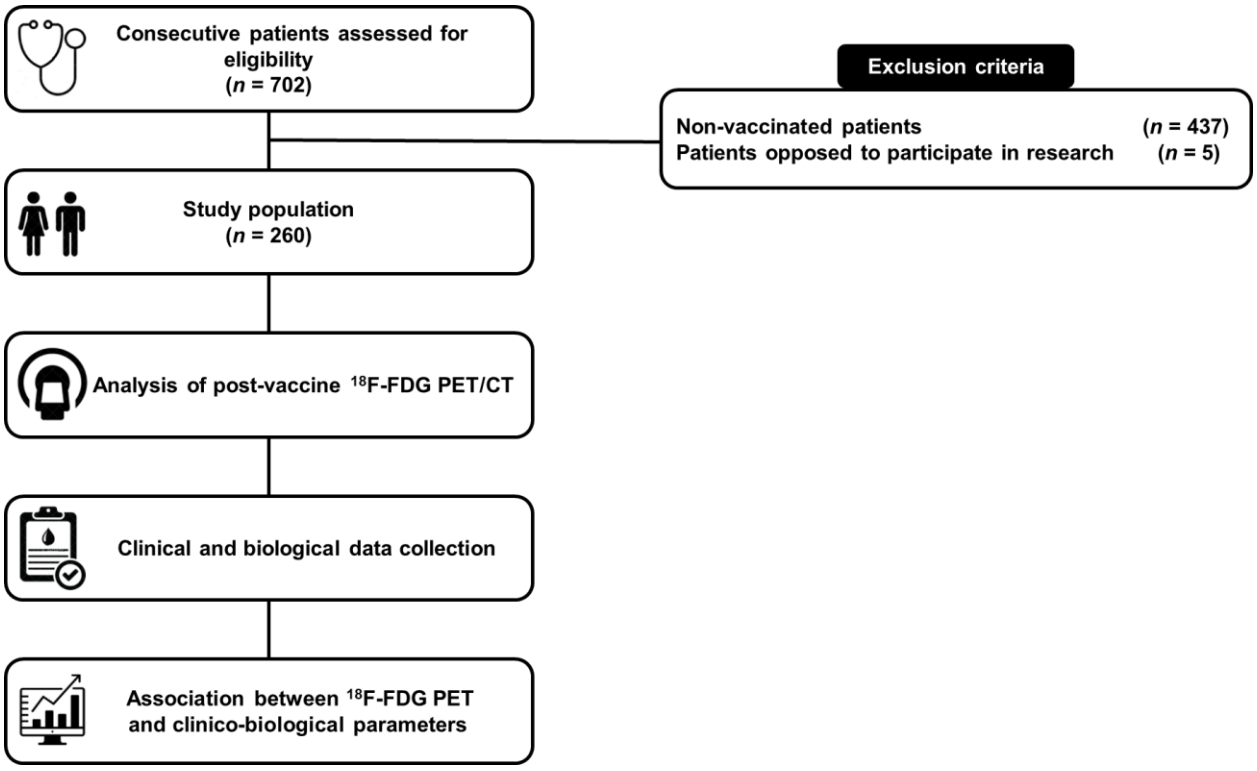
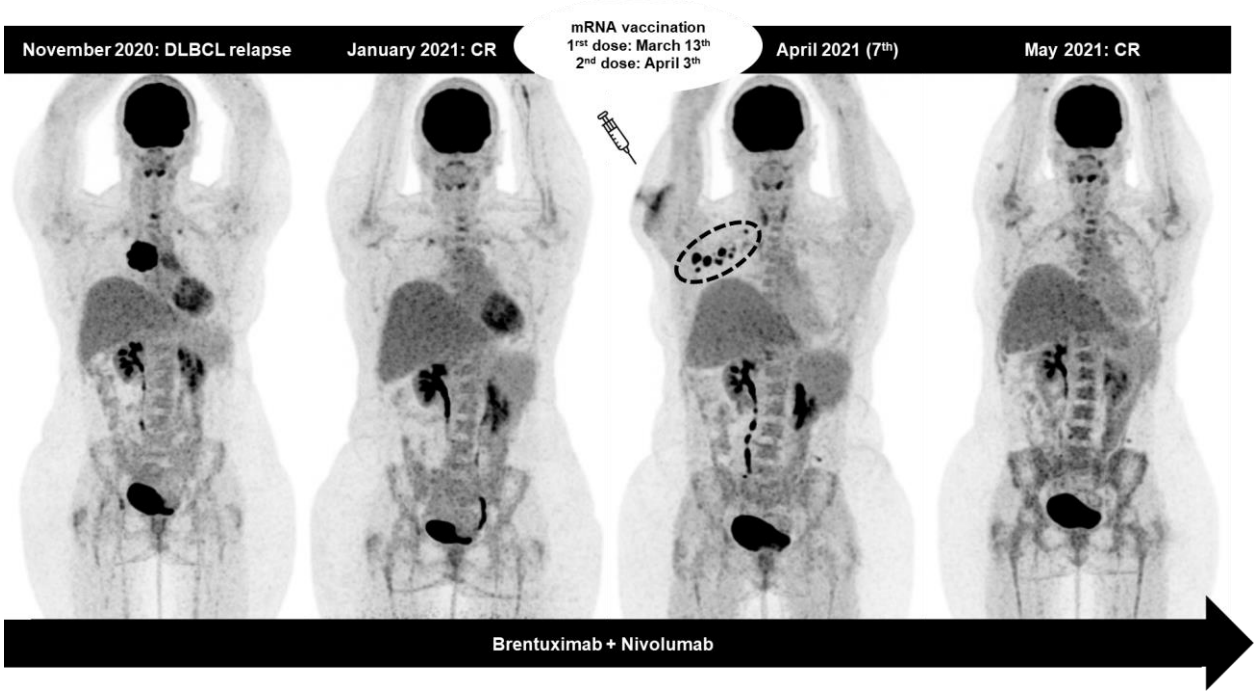
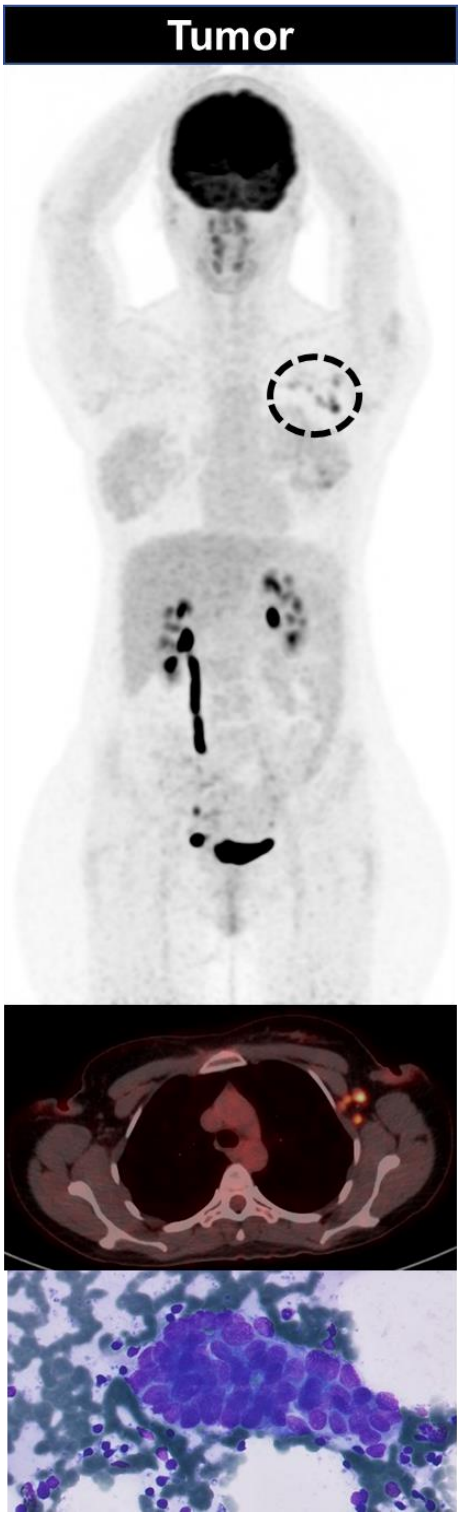
FIGURES

<u>Figure 1:</u> Flow chart.



<u>Figure 2:</u> Illustration with ^{18}F-FDG PET images (MIP) of a patient with relapsed DLBCL.
Figure legend. ^{18}F-FDG PET images of a forty-five-years old woman with relapsed DLBCL in the mediastinum (first MIP). The patient was treated with Brentuximab and Nivolumab and experienced a complete metabolic response at 2 months after the initiation of therapy (second MIP). While continuing the lymphoma therapy, she received two mRNA COVID-19 vaccine injections in the right deltoid. The ^{18}F-FDG PET/CT scan performed four days after the last vaccine dose (third MIP) showed several HLN (black circle). On the subsequent ^{18}F-FDG PET/CT scan performed one month later (fourth MIP), HLN disappeared, strongly suggesting their relation to vaccination. This clinical presentation thus highlights the presence of v-HLN in a patient with less than 50 years and normal values of ALC at the time of ^{18}F-FDG PET/CT, which was performed less than 30 days after the last vaccine dose.
Abbreviations. DLBCL (diffuse large B cell lymphoma), MIP (maximum intensity projection), HLN (hypermetabolic lymph nodes), mRNA (messenger ribonucleic acid), CR (complete response), ALC (absolute lymphocyte count).

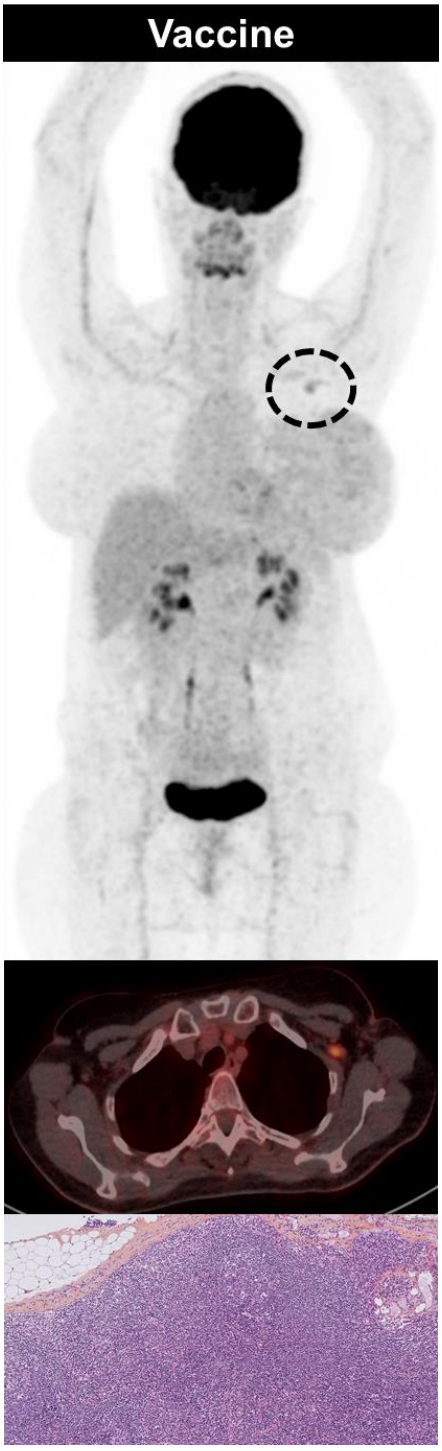




<u>Figure 3:</u> Illustrations using ^{18}F-FDG PET images and histological findings in a patient with early-stage BC patients who received vaccine injection ipsilateral to the tumor.

Figure legend. MIP on the first line showing HLN in the left axilla. Fused axial ^{18}F-FDG PET/CT images on the second line also showing left axillary HLN. Pathological findings showing tumor cells on fine-needle aspiration cytology (MGG staining, zoom X20).

Abbreviations. BC (breast cancer), MIP (maximum projection intensity), HLN (hypermetabolic lymph nodes), MGG (May-Grünwald/Giemsa).



<u>Figure 4:</u> Illustrations using ^{18}F-FDG PET images and histological findings in a patient with early-stage BC patients who received vaccine injection ipsilateral to the tumor.

Figure legend. MIP on the first line showing HLN in the left axilla. Fused axial ^{18}F-FDG PET/CT images on the second line also showing left axillary HLN. Pathological findings showing benign reactive changes on sentinel lymph node biopsy (right, HES coloration, zoom X5).

Abbreviations. BC (breast cancer), MIP (maximum projection intensity), HLN (hypermetabolic lymph nodes), HES (hematoxylin-eosin-saffron).

TABLES

Table 1: Patient's characteristics.
Abbreviations. mRNA (messenger ribonucleic acid), ALC (absolute lymphocyte count), HLN (hypermetabolic lymph nodes), SUVmean (mean standardized uptake value), SUVmax (maximum standardized uptake value), SLR (spleen-to-liver ratio).

	ALL PATIENTS n=260	BREAST CANCER PATIENTS n=145
	Number (%), median [range]	
DEMOGRAPHIC CHARACTERISTICS		
Age (years)	50 [23-96]	67 [28-95]
Sex (female)	209 (80)	145 (100)
VACCINE		
mRNA vaccine (versus viral vector vaccine)	233 (90)	128 (88)
2 doses (versus 1 dose)	110 (42)	61 (42)
Delay in days between first dose and PET (patients who had 1 dose)	14 [1-51]	13 [1-44]
Delay in days between second dose and PET (patients who had 2 doses)	23 [1-67]	24 [1-67]
DISEASE		
Breast cancer (early versus advanced stage)	145 (56)	54 (37) versus 91 (63)
Hematological malignancy (lymphoma, leukemia, myeloma)	39 (15)	na
Thoracic cancer	17 (7)	na
Digestive cancer	15 (6)	na
Gynecologic cancer	14 (5)	na
Head and neck cancer	14 (5)	na
Other types of cancer (thyroid, sarcoma, melanoma)	11 (4)	na
Non-oncologic indications (inflammatory or infectious diseases)	5 (2)	na
TREATMENT		
No specific treatment	106 (41)	47 (32)
Endocrine therapy	28 (11)	24 (16.5)
Chemotherapy	46 (17.5)	25 (17.5)
Targeted therapy	18 (7)	9 (6)
Immune checkpoint inhibitors (single-agent or combined therapies)	8 (3)	0 (0)
Endocrine therapy + targeted therapy	39 (15)	37 (26)
Chemotherapy + targeted therapy	4 (1.5)	3 (2)
Steroids (>10 mg of prednisone-equivalent dose 10mg)	12 (5)	5 (3)
PERIPHERAL BLOOD		
Delay between blood analysis and PET (days)	2 [0-28]*	3 [0-28]†,
ALC (G/L)	1.4 [0.3-18.3]*	1.5 [0.4-5.0]†,
Lymphopenia	74 (44)*	41 (39)#
HLN on ^{18}F-FDG PET/CT		
Delay between the last vaccine injection and PET (days)	17 [0-67]	19 [0-79]
Etiology: vaccine-induced/tumor-related/indeterminate/none	90 (35)/14 (5)/6 (2)/150 (58)	56 (37)/6 (4)/6 (4)/77 (53)
HLN SUVmax	3.7 [2.0-26.3]	3.7 [2.0-26.3]

*Note: *n=170pts; †, n=106pts.*

<u>Table 2:</u> Parameters associated with v-HLN in the whole cohort (univariate and multivariate logistic regression analyses).

Abbreviations. v-HLN (vaccine-induced hypermetabolic lymph nodes), OR (odds ratio), CI (confidence interval), mRNA (messenger ribonucleic acid).

PATIENTS: n = 260	**LYMPH NODE(S) v-HLN**			
	Univariate		**Multivariate**	
Variable	p	OR (CI 95%)	p	OR (CI 95%)
AGE				
≥ 50 years	0.02	1 (reference)	0.04	1 (reference)
< 50 years		2.4 (1.2-4.9)		2.2 (1.0-4.5)
IMMUNOSUPPRESSION				
Yes	0.86	1 (reference)	-	-
No		0.95 (0.5-1.7)		-
LYMPHOPENIA				
Yes		1 (reference)		1 (reference)
No	0.04	1.9 (1.0-3.8)	0.03	2.2 (1.1-4.3)
Unknown	0.38	1.4 (0.7-2.7)	0.37	1.4 (0.7-2.8)
TYPE OF VACCINE				
mRNA	0.66	1 (reference)	-	-
Viral vector		1.2 (0.5-2.7)		-
NUMBER OF VACCINE DOSE(S)				
1	0.45	1 (reference)	-	-
2		1.2 (0.7-2.0)		-
DELAY BETWEEN THE LAST INJECTION AND PET				
≥ 30 days	0.02	1 (reference)	0.01	1 (reference)
< 30 days		2.3 (1.2-4.9)		2.6 (1.3-5.6)

<u>Table 3:</u> Parameters associated with v-HLN in BC patients (univariate and multivariate logistic regression analyses).

Abbreviations. v-HLN (vaccine-induced hypermetabolic lymph nodes), BC (breast cancer), OR (odds ratio), CI (confidence interval), mRNA (messenger ribonucleic acid).

PATIENTS: n = 145	**LYMPH NODE(S) v-HLN**			
	Univariate		**Multivariate**	
Variable	p	OR (CI 95%)	p	OR (CI 95%)
AGE				
≥ 50 years	0.17	1 (reference)	-	-
< 50 years		1.9 (0.7-4.9)		-
IMMUNOSUPPRESSION				
Yes	0.26	1 (reference)	-	-
No		1.2 (0.9-1.9)		-
LYMPHOPENIA				
Yes		1 (reference)		1 (reference)
No	0.04	2.5 (1.1-6.1)	0.02	2.9 (1.2-7.4)
Unknown	0.19	1.9 (0.8-6.7)	0.16	2.0 (0.8-6.9)
TYPE OF VACCINE				
mRNA	0.90	1 (reference)	-	-
Viral vector		0.9 (0.3-2.7)		-
NUMBER OF VACCINE DOSE(S)				
1	0.55	1 (reference)	-	-
2		0.8 (0.4-1.6)		-
DELAY BETWEEN THE LAST INJECTION AND PET				
≥ 30 days	0.12	1 (reference)	0.06	1 (reference)
< 30 days		2.0 (0.9-4.8)		2.3 (0.9-6.3)

Table 4: Early-stage BC patients with vaccination ipsilateral to the tumor.
Abbreviations. mRNA (messenger ribonucleic acid), LN (lymph nodes), SUVmax (maximum standardized uptake value), ALC (absolute lymphocyte count), unk (unknown).

PATIENTS: n=7	**#1**	**#2**	**#3**	**#4**	**#5**	**#6**	**#7**
Demographic characteristics							
Age (years)	45	52	38	81	48	76	66
Breast tumor	left	left	left	left	left	right	right
Vaccination							
Injection site (deltoid)	left	left	left	left	left	right	right
Type	mRNA	mRNA	mRNA	mRNA	viral vector	viral vector	mRNA
Number of doses	1	1	1	2	1	1	2
^{18}F-FDG PET/CT imaging							
Delay last dose - PET (days)	7	3	11	22	32	6	17
Hypermetabolic LN	left axilla	left axilla	left axilla	left axilla	left axilla	right axilla	right axilla
LN SUVmax	7.9	2.0	3.2	4.3	3.7	3.4	4.3
LN size (small axis in mm)	11	7	8	6	8	6	12
Nb of hypermetabolic LN	> 5	> 5	> 5	4	2	4	> 5
Peripheral blood							
ALC (G/L)	1.92	unk	2.35	1.70	0.84	unk	2.32
Lymphopenia	no	unk	no	no	yes	unk	no
Histology							
Modality	cytology	cytology	sentinel LN	sentinel LN	sentinel LN	none	cytology
ETIOLOGY	**TUMOR**	**TUMOR**	**VACCINE**	**VACCINE**	**VACCINE**	**UNKNOWN**	**TUMOR**

GRAPHICAL ABSTRACT