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Original Article

Perspectives of positron emission tomography with 2-deoxy-2-fluorine-18-flu-d-glucose (18fdg-pet) in predicting the histological response of high grade epithelial ovarian cancer to neoadjuvant chemotherapy. A primary descriptive study

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Abstract: Ovarian cancer (OC) was most often diagnosed at an advanced stage due to poor symptoms, the lack of an effective screening method and the limitations of examinations for the early detection of the disease. Sadly, the surgical approach is not possible for all patients, therefore the neoadjuvant chemotherapy (NACT) was a good standard approach before the complete cytoreduction. Currently we don't have indisputable and definitive directives about tools to predict the anatomopathological response. This study investigates the potential of 18FDG-PET in predicting the histological response in high grade epithelial OC before NACT treatment. Ten advanced primarily inoperable OC patients treated with NACT were recruited into this prospective study, then 4 patients could be eligible to analyze, with 12 target lesions eligible to comparative analyses with 18FDG-PET against histological analyses. All patients after NACT presented clinical and biological responses, with normalization of CA 125. Four patients, with 12 resections specimens identified to corresponding 18F-FDG-PET/CT imagery, had showed concordant aspects of response. This preliminary study has demonstrated the role in predictive response of 18FDG-PET, but the complexity in comparative analyses of morpho-functional imagery and histological study includes many difficulties. Thus, to obtain statistically robust results, a large-scale study must be conducted, based on these very encouraging results.

Keywords: Ovarian cancer, 18FDG-PET, neo-adjuvant treatment, cell viability, CRS, CA 125, CA-125 KELIM

Introduction

Ovarian cancer (OC) can be defined as an uncontrolled proliferation of initially normal ovarian cells as a result of genomic mutation [1]. In 2020, ovarian cancer accounted for 3.4% of cancers and 4.7% of cancer-related deaths in women [2]. Diagnosis is most often made at an advanced stage due to poor symptoms, the lack of an effective screening methods, and the limitations of examinations for the early detection of the disease [1]. The 5-year survival rate for women with ovarian cancer is estimated at 30-50% for all stages and 20-25% for advanced stages [3]. Approximately 60% of OC are diagnosed at advanced-stage disease [4].

Disease prognosis depends on achieving complete cytoreduction at upfront primary debulking surgery (PDS) [5]. Unfortunately, this is not possible for all patients, therefore the neoadjuvant chemotherapy (NACT) is a good alternative treatment which can, in most cases, increases the probability of complete cytoreduction followed by interval debulking surgery (IDS) [6, 7], often reducing the invasiveness of surgery resection [8]. Considering that macroscopic or microscopic complete resection surgery it is not always achieved, so after NACT followed by IDS, it must be completed with adjuvant chemotherapy [9].

NACT/IDS is the most recommended and usual approach for advanced OC [10-12], consequentially it remains strongly necessary to have tools to evaluate NACT's response. Dosing of carcinoma antigen 125 (CA-125) is routinely recommended to evaluate response to treatment but should always be combined with morphological and clinical evaluation [10]. Human epididymis protein 4 (HE4) was a promising new biomarker, but currently not widely spread in clinical practice due to a relatively high cost and limited access [8]. However, model CA125 KELIM™ which is easy to use and quite unexpensive, index for platinum-based chemotherapy efficacy, CA-125 KELIM™ is calculated with at least 3 CA-125 values measured within the first 4 cycles of NACT [13, 14].

From an histological point of view, these last years, the CRS score was validated for standardization of the assessment of the response after neoadjuvant chemotherapy for high-grade OC [15]. This histological score has three levels: CRS 1 meaning the absence or minimal tumor response, with mainly viable tumor with no or minimal regression-associated fibroinflammatory changes, limited to a few foci, CRS 2 meaning partial tumor response amid viable tumor that is readily identifiable and CRS 3 meaning complete or near-complete response with no residual tumor or minimal irregularly scattered tumor foci seen as

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individual cells, cell groups, or nodules up to 2 mm maximum size [15, 16].

From the imaging point of view, several exams may bring information helping in the assessment of the treatment response.

A study (Bregar *et al.*) investigated the performances of computed tomography in predicting the optimal response after NACT for cytoreductive surgery. Bregar et al., had demonstrated that the modification of score before and after neoadjuvant chemotherapy had reduced the variability and predicts surgical outcome [17].

Another study (Engbersen *et al.*) investigated the performance of diffusion-weighted magnetic resonance imaging (DW-MRI) in predicting the feasibility of complete surgery for ovarian cancer. Engbersen et al had demonstrated that DW-MRI is an accurate predicting imaging study to complete cytoreduction, so helpful to predict whether a complete resection or not [18].

According to the recommendations of the European Society of Medical Oncology (ESMO), the preoperative evaluation can be performed by using 18FDG-PET or DW-MRI. This imagery should be ideally completed with a pelvic ultrasound performed by an expert and laparoscopy (5).

Until today, 18FDG-PET has not a clear and validated positioning in early detection of response to treatment in OC patients [19]. Cho *et al.* study demonstrated an interest for 18FDG-PET to estimate tumor perfusion during regional hyperthermic intraperitoneal treatment, but without clinical benefit [20]. In addition, Franceschi *et al.* investigated the performance of the combination of 18FDG-PET and magnetic resonance imaging (MRI) for predicting response to NACT [21]. Moreover, Avril *et al.* had evaluated the performance of the 18FDG-PET for predicting the response to NACT in terms of overall survival [22]. In 2015, Valllius *et al.* evaluated the performance of the PET in predicting the histological response to NACT according to the degenerative changes detectable in residual tumor and surrounding stroma [23].

Therefore, the purpose of this study is to assess the potential of 18FDG-PET in predicting the histological response in high grade epithelial OC before NACT treatment, comparing residual 18FDG-PET positive areas with correspondent histological analysis.

Material and methods

Ten advanced, primarily inoperable OC patients treated with NACT were enrolled into this prospective study at the Department of Obstetrics and Gynecology of both APHP Louis Mourier et Bichat, Paris, France, from January 1st 2016 to December 31st 2019.

Among the 10 patients, included in this study, only 4 patients could be analyzed. Actually, only for these patients it was possible to identify the corresponding anatomical sites of 18FDG-PET positive area. The other 6 patients presented a complete functional-morphological response to 18FDG-PET, with also pathological complete response (pCR).

Chemotherapy

All patients received a cycles of Paclitaxel 175 mg/m^2 and Carboplatin AUC 5, administered at interval of 3 weeks. 18F-FDG-PET/CT imaging was performed before IDS. A monitoring of CA 125 was performed every 3 weeks before each new cycle of NACT. CA-125 KELIM™ is calculated once the 4 cycles has been achieved. A standard support care was administrated during the cytotoxic treatment.

Surgery

All IDS were performed by board-certified gynaeco-oncologists with over 10 years of post-fellowship experience and assisted by fellows (PE, TN). All IDS were performed with the goal to achieve complete cytoreduction. The IDS procedure consisted of pelvic tumours, omentum, pelvic and paraaortic lymph nodes, and extra-pelvic tumours resection. The IDS procedure was concluded with a visual inspection of the abdominopelvic cavity confirming complete macroscopic resection. As a standard procedure the surgeon proceeds to random biopsies collected from proximal areas free of macroscopic disease for systematic documentation when feasible. During the IDS a surgical resection scheme with numbering and orientation of each single exported anatomical piece is automatically created by the surgical team.

18F-FDG-PET/CT

The whole-body 18F-FDG-PET/CT was performed with either 64-row Discovery STE or VCT (General Electric Medical Systems, Milwaukee, WI, USA). The 18F-FDG-PET/CT imaging protocol foreseen that patient were fasted for 6 h prior to 18F-FDG-PET/CT. The examination starts with glucose level below 144 mg/dl at the time of 2-[18F]FDG injection (weight-based: weight (kg)×0.13 mCi), for an approximate duration of 45-60 min. The targeted lesions were identified from 18F-FDG-PET/CT before and after NACT by a board-certified nuclear medicine physician (KB). Metabolic response to initial treatment as defined by an unequivocally negative 18F-FDG-PET/CT using the PET response criteria in solid tumors (PERCIST) scale was applied for all patients [24, 25].

Histological analyses

Histological analyses of all resected specimens at IDS were performed by a board-certified pathologist specialized in gynecological pathology with over 10 years post-fellowship (MB). MB examined all tissue specimens, iden-

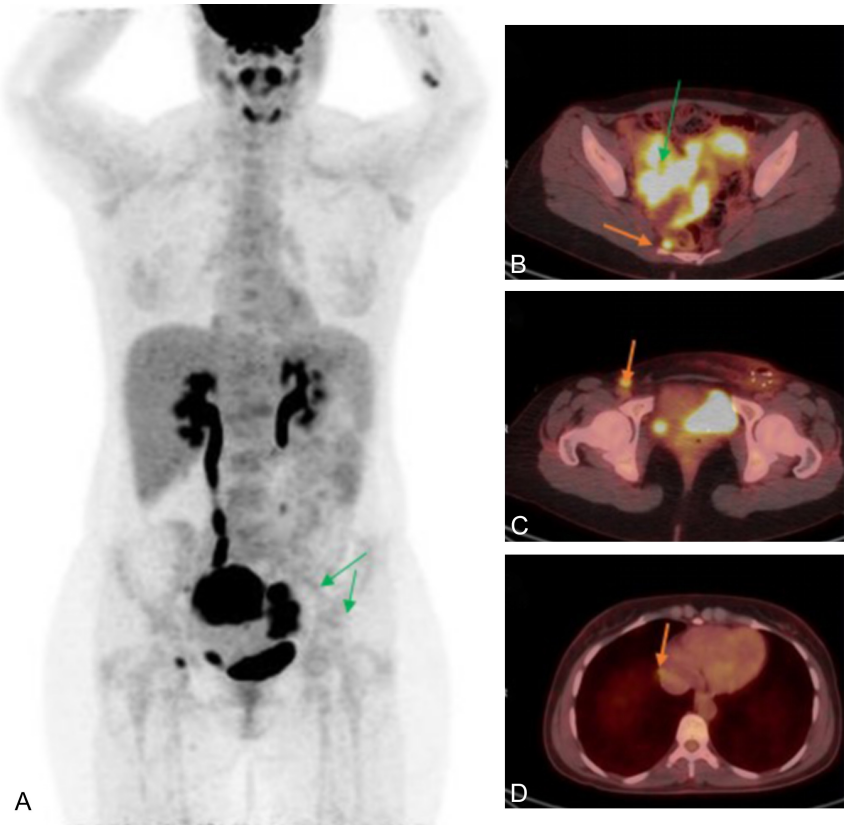

Figure 1. Illustrates respectively intense 18F-FDG-PET/CT baseline uptake on ovarian mass, green arrow, SUV Max 12,4 (A, B), lymph nodes (orange arrows). Right parasigmoid lymphadenopathy, SUV Max 6 (C). Right parieto-colic groove behind the liver lymphadenopathy, SUV Max 2,5 (D).

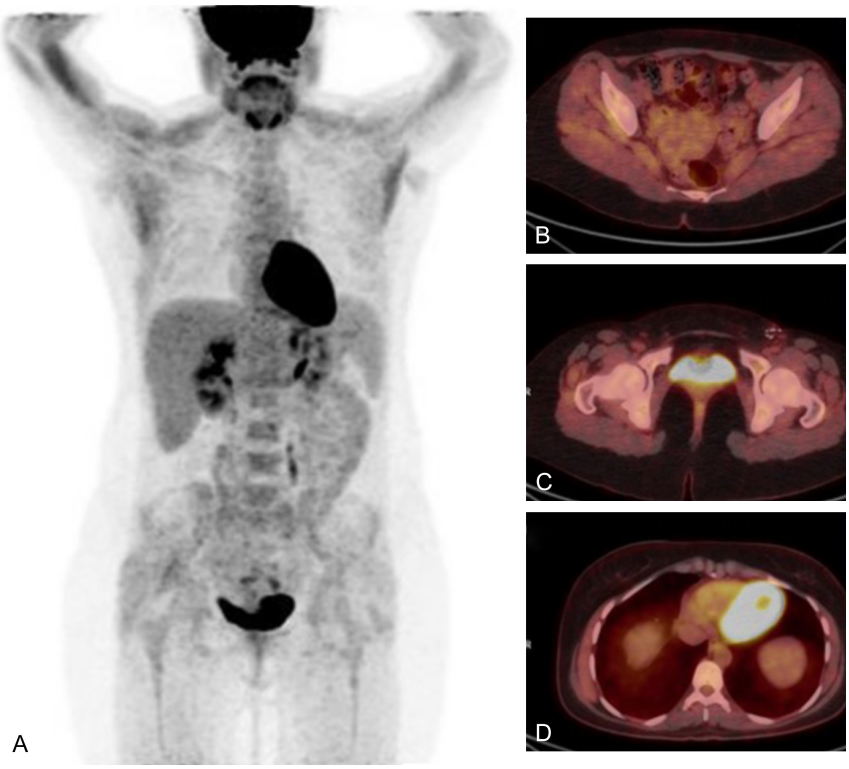

Figure 2. Illustrates a complete metabolic response after NACT. This favorable response is confirmed by histology.

tified the anatomical district of origin, with the help of the operating scheme, and graded the response to NACT based on CRS [15, 16]. MB quantified the residual viability tumoral cells percentage. All anatomical findings were catalogued, classified and analyzed according to the surgical scheme created at the time of IDS. CRS analyses were carried out for all resected specimens.

Histological/imaging analyses comparison

Once done, KB and MB were able to compare the 18F-FDG-PET/CT imagine before and after NACT with identified corresponding removed tissue. This was made possible thanks to a precise geographical description of the anatomical removal carried out by the gynecological surgery team. They (KB and MB) had identified for each patients the 18F-FDG-PET radioisotope glucose signal. Then, with the help of peri-operatory scheme, they spotted the corresponding positive area of 18F-FDG-PET, before and after NACT. KB identified the radioisotope SUVMax index before (**Figure 1**) and after NACT (**Figure 2**) of 18F-FDG-PET. MB analyzed this radioisotope positive area with the quantification of residual viability tumoral cells percentage and calculating the CRS score (**Figures 3** and **4**).

Statistical analysis

This study is mainly descriptive due to the small number of patients. Wilkoxon Non-Parametric Tests and Fisher Exact Test were used to compare different numeric categorical variables. A ROC curve helped to define an indicative threshold for SUVMAV value using the Youden index.

The JMP software was used for statistical analysis. The study was conducted in accordance with the Declaration of Helsinki, the International Conference on Harmonization, harmonized Tripartite Guidelines for good clinical practice, and site-specific institutional review boards approved the study protocol and amendments. The protocol was approved by our local Ethics Committee and our Institutional Review Board. Informed consents were obtained from all patients from the prospective cohort and retrospective cohort.

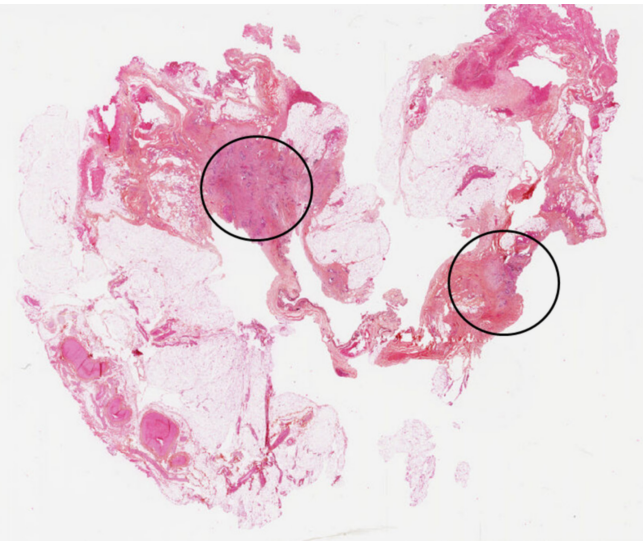

Figure 3. Mesocolic nodule, whole slide image, Hematein, Eosin and Saffron stain. Circles: residual tumoral nodules after NACT.

Results

Patients

From the 10 patients recruited in this study, diagnosed between Avril 2017 and June 2019, four were concerned by the histological/imaging comparison. The median age of 4 patients was 60 and was not significantly different from the 6 others patients (median: 64). No significant differences were observed with other variables (Nb of cycles, Baseline CA125, Pre-IDS CA125 and Kelim index) (**Table 1**).

For the 4 patients, the FIGO stage was IIIB. They received NACT, without dose reduction for all cycles, with good tolerability. Prior to each NACT cycle a dosage of CA 125 was automatically for all patients. CA 125 median before starting NACT was 400 UI/L, although 1 patient with a very high level (1100 UI/L), which came back to normal after the last NACT cycle (13 UI/L) (**Table 1**).

The 18F-FDG-PET/CT imaging protocol was performed between 1 to 7 days before initiating NACT and 20-22 days after the fourth cycle of NACT. All patients presented, after 4 cycles NACT, clinical, biological and metabolic response, so they received a complete IDS after 4 weeks of the fourth cycle NACT.

Assessment of chemotherapy response

All patients after NACT presented clinical and biological response, with CA 125 normalization. The KELIM index median was 0.96 and for all patients were <1. All patients showed a complete response to PERCIST criteria at 18F-FDG-PET/CT after the last cycle of NACT, 4 weeks before IDS. All patients present a biological response with a CA 125 normalization (with a 97% decrease).

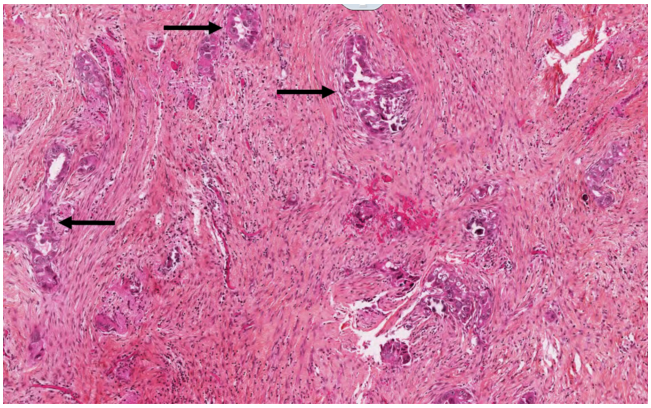

Figure 4. The mesocolic nodule, G5 magnification, Hematein, Eosin and Saffron stain. Peritoneal localization of tumoral glands in a desmoplastic stromal reaction (arrows).

Imaging/anatomy study

The 18F-FDG-PET/CT with the surgical plan support made it possible for KB and MB to find out the imaging 18F-FDG-PET/CT matching the resected specimens at IDS. Regarding these 4 patients, 12 resection specimens with a 18F-FDG-PET/CT imaging match.

Each patient's data were analyzed for each resection specimen respectably (**Table 2**).

The SUVMax median for patient was 5.12 (range 2.8-7.9) before NACT vs 1.47 (range 0.76-2.63) after NACT referring respectively to a decrease of 89.7%, 89.1%, 76.9% and 73.4% for the four patients. If we consider the 12 resection specimens the median SUVMax was before NACT 4.5 (range 1.7-12.4) vs 1.1 (range 0-3.7) after NACT. The median evolution was -77% (range: -100% to +19.4%). If we consider these evolutions as a tool to predict the complete histological response analyzed by pathologists, the threshold estimated from the ROC curve is approximatively 75%.

Discussion

Recently, Lee et al demonstrated no difference versus 18FDG-PET compared with contrast-enhanced CT, nevertheless the higher metabolic SUVmax was associated with chemotherapy response score (CRS), showing capacity for 18FDG-PET to predict the histopathological non-responders [26].

Currently the position, in clinical practice of 18FDG-PET was limited to the evaluation of recurrent disease when other conventional diagnostic tools were negative or non-conclusive [27]. Actually, like for every functional imaging technique, we are limited by the standardization and reproducibility of these techniques which may present many variabilities from one device to another and from one operator to another [28]. Therefore, these techniques have not been adopted yet in clinical trials and in clinical guidelines. However, it seems to be an obvious and precious contribution for those practicing cancerology in diagnostic and therapeutic predictive response.

Table 1. Demographics and clinical data of patients

Demographic characteristics	Analyzed patients	Not analyzed patients
N	*4*	*6*
Age (years)	*37, 60, 66, 60*	*66, 74, 58, 62, 66, 38*
FIGO IIIB	4	6
No. cycles of NACT before IDE	4, 7, 4, 3	*4, 4, 6, 4, 3, 3*
CA-125 (UI/L)		
Baseline	*350, 256, 1100, 450*	*876, 370, 120, 1200, 798, 550*
Pre-IDS	*12, 9, 14, 21*	*30, 22, 8, 24, 24, 13*
HRD/BRCA (Y/N)	*0/4*	*0/4*
KELIM	*0.96, 0.98, 0.95, 0.94*	*0.96, 1.1, 0.93, 0.77, 0.76, 0.75*
Unfavorable (<1)	*4/4*	*5/6*
DCD	1/4	0/6

Table 2. Histological observation of 4 patients

Patient	Resection's specimens	18F-FDG-PET/CT (SUV Max Baseline)		18F-FDG-PET/CT (SUV Max pre-operatory)		Δ SUV Max		Residual tumour anatomopathology		CRS
		Target	Median patient	Target	Median patient	Target	Med patient	Target	Median patient	
1	Ovarian mass	12.4	5.4	1.8	0	85.48%	100%	40%	0%	2
	Right inguinal lymphadenopathy	5.4		2		62.96%		0%		
	Right parasigmoid lymphadenopathy	6		0		100.00%		0%		
	Right cardiophrenic lymphadenopathy	2.4		0		100.00%		0%		
	Right parieto-colic groove behind the liver lymphadenopathy	2.5		0		100.00%		0%		
2	Hilum lymphadenopathy	3.1	3.1	3.7	3	-19.35%	16.7%	0%	0%	2
	Mediastino-hilar lymphadenopathy	3.6		3		16.67%		0%		
	Left internal mammary lymphadenopathy	1.7		1.2		29.41%		0%		
3	Right axillary lymphadenopathy	5.8	5.3	0.5	1	91.38%	81.1%	0%	0%	2
	Left axillary lymphadenopathy	2.4		1		58.33%		0%		
	Carcinomatosis nodule	5.3		1		81.13%		0%		
4	Laterocaval and inter-aortico-cave at the level of the renal hilum lymphadenopathy	7.9	7.9	2.1	2.1	73.42%		0%	0%	3

The 18F-FDG-PET/CT baseline compared to pre-operatory after NACT expressed in SUV Max and histology analyses, CRS and tumor cytological residual of IDS, compared to 18F-FDG-PET/CT pre-operatory after NACT.

Our study showed that 18FDG-PET was unable to detect a low histological residual tumor. Thus, it doesn't permit very high sensitive and selections. Therefore, 18FDG-PET was informative about the therapeutic response, matching with biological index (CA 125 and KALIM index).

This therapeutic response does not allow to differentiated the complete response from the partial histological response, especially regarding aggressive forms of ovarian adenocarcinoma, with more targets high SUVMax and/or with high CA 125.

The biggest limitation of our study was the number of patients and targets that have been studied and which restricted our statistical analysis. Actually, we primely faced complete disappearance of functional targets following a complete response in 6 out of all our patients. However, these 6 patients also showed a complete anatomopathological response. Then, the comparative study was very complex, requiring an analytical endeavor to check anatomical correspondence from the nuclear medicine physician and the pathologist, without forgetting the mapping work performed by the surgeon, requiring more care and accuracy than in clinical practice.

The 18FDG-PET, in our study, highlighted the functional tumor heterogeneity of 18FDG with SUV Max variable from one patient to another patient, from one lesion to another lesion within the same patient. Infect, in patient 2 the target "Hilum lymphadenopathy" present decrease of SUVMax against the targets "Mediastino-hilar lymphadenopathy" and "Left internal mammary lymphadenopathy" that present an increase of SUVMax. However, the anatomopathological analyses for all 3 targets found no residual tumor. Discordances and heterogeneities represent a limitation of this imaging technique, even more than morphological imaging techniques compared to functional [25, 28].

Moreover, the 18FDG-PET, as all functional and morphological imaging still presents an unmet limit: the absence of a three-dimensional analysis.

This study highlights, on one hand the huge amount of anatomopathological materials to be analyzed by our pathologists in this context and on the other hand the modalities of this histological analysis remining individual and subjective in its selection.

It seems obvious that the 18FDG-PET role could be helpful in suggesting which anatomopathological area to analyses. This would provide an extra qualitative benefit in the analysis of the anatomopathological chemotherapy response, reducing false negatives.

Conclusion

This preliminary study has demonstrated the potential role of 18FDG-PET in predictive response, but the complexity in the comparative analyses of morpho-functional imaging and histological study implies many difficulties. The implementation, of this protocol is complex and requires a large medical investment, but once validated the systematic preoperative 18FDG-PET would improve and ease the work of the pathologist with target anatomopathological analysis and therefore avoiding histological analyses false negatives. However, first, to achieve statistically robust results, a large-scale study must be conducted, based on these very promising results.

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Informed consent was obtained from all individual participants included in the study.

Disclosure of conflict of interest

None.

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