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Increased ^{18}F–choline PET/CT Uptake in Undifferentiated Prostate Cancers with High Proliferation Index

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Abstract

Aim: The main aim of this pilot study was to investigate the possible association among ^{18}F-choline uptake, proliferation index (Ki67 expression), differentiation of cancer cells (vimentin expression) and free PSA serum concentration in 10 prostate cancer patients. To this end, we collected data from patients with a Gleason Group of at least six and/or a PSA level greater than 4 ng/mL underwent to both ^{18}F–choline PET/CT investigation and bioptic procedures. **Methods:** Our data clearly showed the capability of ^{18}F–choline PET/CT to predict some histopathological characteristics of prostate tumors. Indeed, linear regression analysis displayed a positive association between ^{18}F–choline, evaluated in terms of SUVaverage, and both the percentage of Ki67 positive prostate cancer cells and the number of vimentin positive cancer cells. **Results:** Vimentin positive cancer cells are frequently present in undifferentiated prostate cancers. Thus, data here reported suggest a possible role of ^{18}F–choline PET/CT in the identification of undifferentiated prostate cancers. As concern the proliferation index, Ki67 is used by oncologists to choose the most appropriate therapeutic plan. The possibility to establish the proliferation index by in vivo investigations such as 18F–choline PET/CT could introduce an amazing improvement in the management of prostate cancer patients. In fact, the value of ^{18}F–choline uptake could provide to clinicians prognostic and predictive information about the growth of prostate cancers. According to the conflicting data reported in the literature, no association was found between the value of free PSA serum concentration and ^{18}F–choline uptake. **Conclusion:** In conclusion, in this pilot study, for the first time, we propose the use of ^{18}F–choline PET/CT to identify undifferentiated prostate cancer lesions characterized by high proliferation index. These data, if confirmed on large population, can be used to improve the current clinical practices planned for the management of prostate cancer patients.

Keywords: ^{18}F–choline PET/CT; Prostate cancer; Proliferation index; Vimentin; Molecular imaging

Introduction

Prostate cancer represents one of the most frequent tumours in the men in worldwide [1]. Indeed, recent reports estimated that prostate cancer is the third leading cause of cancer-related deaths in both American and European men following lung cancer and colorectal cancer, respectively [1-4]. Prostate cancer frequently occurs in older men with 6/10 being diagnosed in men aged 65 or older with an average age of about 66 at the time of cancer diagnosis [1-4].

Since the high incidence of prostate cancer in older men, diagnostic procedures require a constant improvement by developing new tests and instrumental investigations. As concern the early detection of prostate cancer the prostate specific antigen (PSA) blood test had been used for most previous tests but this test has been critiqued because it may miss some cases of cancer while it may indicate the presence of cancer when prostate cancer could not be found [5].

Indeed, several studies reported pitfalls in the use of PSA serum concentration as an early biomarker for prostate cancer [6-8]. Thus, currently the diagnosis of prostate cancer is based on imaging methods including transrectal ultrasound [9], Magnetic Resonance Imaging (MRI) [10], Computer Tomography (CT) [11] and Positron-Emission Tomography (PET) [11-13]. However, the final diagnosis and classification of prostate cancer lesions is performed by both histological and immunohistochemical analysis [14]. Specifically, the classification methods used in clinical practice is the Gleason Score that classify the prostate lesions according to morphological and microscopical characteristics of tissue architecture and prostate cells [14].

In recent years PET/CT analysis of prostate cancer patients with several radiopharmaceuticals such as ^{18}F-choline (^{18}F–ethylcholine or ^{18}F–methylcholine) [15], ^{11}C–choline PET/CT [16], FDG [17] and PSMA ligand [17,18] acquired a

central role in the management of these patients. In fact, PET/CT investigations can be used for both diagnosis and follow-up of patients affected by prostate cancer with an eminent role in the detection of metastatic lesions [19]. Despite interesting data are reported for all radiopharmaceuticals mentioned above, PET/CT with radiolabeled choline is currently the most used molecular imaging analysis for prostate cancer patients [19]. In fact, several studies have been depicted PET/CT with radiolabeled choline as a useful technique in the management of prostate cancer patients [12, 20,21], especially in relation to absolute PSA and PSA kinetics value at the time of the scan [22]. Specifically, Calabria et al. reported that ^{18}F-choline PET/CT can help identify early recurrences, even in the case of low PSA levels (<1 ng/mL) [12].

In addition, we recently demonstrated that ^{18}F-choline PET/CT is able to detect prostate cancer lesions with high propensity to form bone metastasis by comparing imaging data, in term of choline uptake, and histological characteristics of bioptic samples [23]. This study supports the idea that ^{18}F-choline uptake can predict some histopathological characteristics thus providing prognostic information capable to improve the management of prostate cancer patients.

Starting from these considerations, the main aim of this pilot study was to investigate the possible association among ^{18}F-choline uptake, proliferation index (Ki67 expression), differentiation of cancer cells (vimentin expression) and free PSA serum concentration in 10 prostate cancer patients.

Methods

Patients

In this study we collected data from patients with a Gleason Group of at least six and/or a prostate-specific antigen (PSA) level greater than 4 ng/mL. The exclusion criteria were a second cancer and neoadjuvant hormonal or radiation therapy prior to surgery. All patients underwent ^{18}F–choline PET/CT analysis 15 to 30 days before MRI-guided biopsies. The day of ^{18}F–choline PET/CT investigation free PSA serum concentration was assessed.

According to inclusion and exclusion criteria, we retrospectively collected data from 10 prostate cancer patients (70.40 ± 1.73 years; range 64–79 years) underwent to both ^{18}F–choline PET/CT analysis and bioptic procedure.

Histological and immunohistochemical studies were performed on each bioptic sample.

The study was approved by the Institutional Ethical Committee of the "Policlinico Tor Vergata" (reference number # 129.18). Experimental procedures were performed in agreement with The Code of Ethics of the World Medical Association (Declaration of Helsinki). All patients have signed an informed consent prior to surgical procedures.

^{18}F–Choline PET/CT analysis

Prostate cancer patients were subjected to ^{18}F–methylcholine (^{18}F–choline) PET/CT analysis. The standardized uptake value (SUV) of the target lesion(s) was measured as previously described [24]. Specifically, we evaluated ^{18}F–choline uptake in the prostate semi-quantitatively using SUVmax and SUVaverage (SUVav) (applying volumes of interest (VOI) with a threshold of 50%) derived from attenuation-corrected PET emission data. However, to reduce the operator-dependent variables, only the values of SUVav were showed in this study. SUVav values were collected to verify a possible correlation among ^{18}F–choline uptake in prostate lesions, the Gleason score, *in situ* expression of prognostic biomarkers (vimentin and Ki67) and free PSA serum concentration values.

MRI-guided biopsies

All patients underwent 1.5- or 3-T MRI before prostate biopsy with or without an endorectal coil. Suspicious lesions at MRI were submitted to a targeted biopsy with the use of real-time TRUS guidance using a software registration system. At least two cores were taken for each suspicious/target lesion. A correspondence between MRI target regions and uptake of choline was observed. All patients underwent a concomitant systematic biopsy at the time of the targeted biopsy, with at least six random cores taken outside the targeted biopsy area.

Histology

After fixation in 10% buffered formalin for 24 hours, prostate tissues were paraffin embedded. Three/four-micrometers thick sections were stained with hematoxylin and eosin (H&E), and the diagnostic classification was blindly performed by two pathologists.

Immunohistochemistry

Immunohistochemical investigations were performed to study the proliferation index of prostate tissues (Ki67) and the expression of a biomarkers involved in the cancer progression (vimentin). Briefly, antigen retrieval was performed on 3 µm-thick paraffin sections by using Citrate pH 6.0 (Ki67) or EDTA citrate pH 7.8 (vimentin) buffers (95 °C for 30 min). Then, primary antibodies Ki67 (rabbit monoclonal antibody clone 30-9, pre-diluted Ventana, Tucson, AZ, USA) and vimentin (mouse monoclonal clone V9; pre-diluted Ventana, Tucson, AZ, USA) were incubated for 1 hour at room temperature. An HRP-DAB Detection Kit (UCS Diagnostic, Rome, Italy) was used to reveal the reaction. An immunohistochemical signal was assessed independently by two investigators by counting the number of positive prostate cancer cells (out of a total of 500 in randomly selected regions).

Statistical analysis

Statistical analysis was performed by using GraphPad Prism 5 Software (San Diego, CA, La Jolla, CA, USA). Linear regression analyses were performed to assess the association among ^{18}F–choline uptake in term of SUVav, expression of Ki67, expression of vimentin and PSA serum concentration values.

Results

Histological Classification

According to the WHO 2016, collected prostate biopsies were classified in acinar adenocarcinomas [14]. Specifically, lesions were evaluated according to the Gleason Group (GG) classification [14]. For each patient, the highest value of GG found in biopsies of target regions has been used. As concern

the GG, we observed 2 patients with GG = 3 + 3, 3 patients with GG = 4 + 3, 2 patients with GG = 4 + 3, and 3 patients with GG = 5 + 4.

^{18}F–Choline PET/CT analysis

PET/CT Analysis analyses showed ^{18}F–Choline uptake in all patients (SUVav max 6.20; min 1.13). No significant differences were observed by comparing both SUVav and GG classification (data no shown) and SUVav and age (r^2 = 0.081; p = 0.8047).

Linear rregression analysis between ^{18}F–Choline uptake and *in Situ* biomarkers

Linear regression analyses have been performed to investigate the possible association between ^{18}F–choline uptake (SUV average) and the expression of both Ki67 and vimentin.

As concern the vimentin expression, linear regression analyses showed a positive and significant correlation between the number of vimentin-positive prostate cancer cells and ^{18}F–choline uptake evaluated in terms of SUV average (r^2 = 0.6970; p=0.0027). Similarly, significant association was observed considering the expression of Ki67 evaluated in terms of percentage of cancer positive cells (r^2 = 0.5786; p=0.0106).

Linear regression analysis between ^{18}F–Choline uptake and PSA serum concentration

The linear regression analysis has been performed to investigate the possible association between ^{18}F–choline uptake (SUV average) and the values of PSA serum concentration.

Our data showed no significant association between SUV average and PSA serum concentration (r^2 = 0.0005; p=0.9472). It is interesting to note that same patients with high SUV average values showed exceptionally low values of free PSA serum concentration (Table 1).

Patients	Age	Gleason Group	Choline Uptake	Tumor Size	Ki67	Vimentin	PSA
1	79	5+4	6.2	1.8	85	289	3.3
2	71	4+3	2.67	1.6	18	99	2.67
3	66	3+4	3.21	0.8	66	190	3.69
4	75	3+3	4.1	1.5	80	201	1.62
5	66	5+4	3.1	1.1	65	152	1.11
6	68	4+3	5.1	1.1	75	305	0.7
7	72	4+3	4.31	8.9	70	220	2.14
8	78	3+3	1.13	1.2	25	154	1.14
9	64	5+4	5.3	2.2	69	189	0.55
10	65	3+4	5.35	1.4	65	287	1.83

*choline uptake (SUVav); Tumor size (cm); Ki67 (%); Vimentin (positive cells/500); PSA (ng/ml)

Table 1: Baseline characteristics of patients.

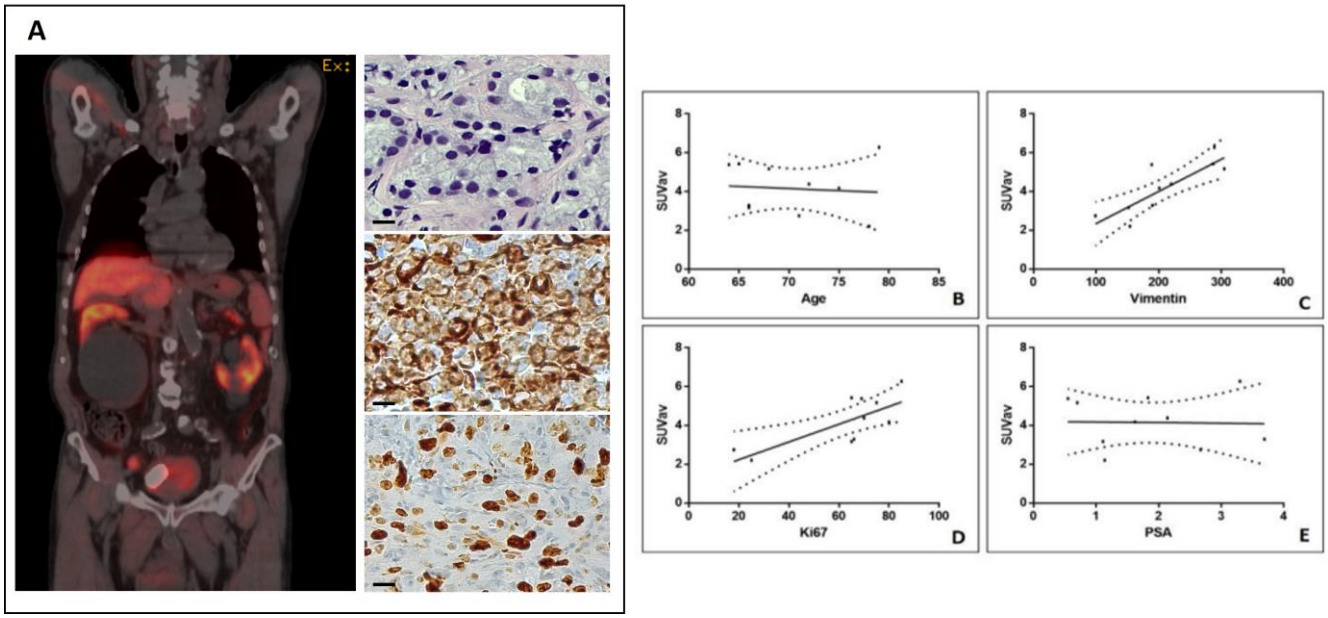

Figure 1: Comparison among ^{18}F–choline uptake, age, histopathological features and free PSA serum concentration. A) ^{18}F–Choline PET/CT maximum-intensity projection in a 69-year-old prostate cancer patient. Bioptic samples showed a 5+4 Gleason Group prostate cancer lesions expressing high value of vimentin and Ki67. (scale bar represents 100 µm for all images) B) Graph displays no association between SUV average and patients' age. C) Graph displays the positive association between SUV average and the number of vimentin-positive cancer cells. D) Graph displays the positive association between SUV average and the number of Ki67-positive cancer cells. E) Graph displays no association between SUV average and free PSA serum concentration.

Discussion

The identification of new diagnostic tools for both early detection and follow-up of prostate cancer lesions, as well as the re-evaluation of already established diagnostic procedures, represent one of the most important scientific aim of the bio-medical research. Indeed, the advent of precision medicine requires a constant research of biomarkers capable to provide prognostic and predictive information by both ex vivo and in vivo analysis.

In this scenario, several groups focused their studies on the identification of new biomarkers capable to improve the current diagnostic path for prostate cancer patients thus reducing its incidence in older men and the relative health costs. However, to reach this goal multidisciplinary approaches are needed. In particular, this can be achieved by developing a strictly collaboration between nuclear medicine and pathology departments as recently suggested by Schillaci et al. [25-27]. Nevertheless, at the state of art, only few studies have been published about the association among imaging and histopathological data of prostate cancers.

Starting from these considerations, the main aim of this pilot study was to investigate the possible association among ^{18}F-choline uptake, proliferation index (Ki67 expression), differentiation of cancer cells (vimentin expression) and free PSA serum concentration in 10 prostate cancer patients.

To this end, we collected data from patients with a Gleason Group of at least six and/or a PSA level greater than 4 ng/mL underwent to both ^{18}F–choline PET/CT investigation and bioptic procedures.

Our data clearly showed the capability of ^{18}F–choline PET/CT to predict some histopathological characteristics of prostate tumors. Indeed, linear regression analysis displayed a positive association between ^{18}F–choline, evaluated in terms of SUVav, and both the percentage of Ki67 positive prostate cancer cells and the number of vimentin positive cancer cells.

The expression of vimentin in prostate cancer cells is considered a sign of the known phenomenon called epithelial to mesenchymal transition [28]. The occurrence of this phenomenon in prostate cancers, and in general in epithelial cancers, is one of the molecular mechanisms involved in tumors progression [29]. In particular, cancer cells that express vimentin filaments acquire the capability to invade surrounding tissues and develop metastatic lesions [30]. In addition, vimentin positive cancer cells are frequently present in undifferentiated prostate cancers. Thus, data here reported suggest a possible role of ^{18}F–choline PET/CT in the identification of undifferentiated prostate cancers. Large cohort population studies could be useful to identify the values of ^{18}F–choline uptake associated to the presence of undifferentiated lesions.

Also, linear regression analysis showed a significant association between the proliferation index (Ki67 expression) and ^{18}F–choline uptake. Ki67 is an *in situ* biomarkers that identify cells in active proliferation phase [31]. Currently, this biomarker is investigated by immunohistochemical reactions to define the proliferation index of several neoplasia such as prostate, breast and melanoma [32,33]. The value of Ki67 is frequently used by oncologists to choose the most appropriate therapeutic plan [32]. The possibility to establish the proliferation index by in vivo investigations such as ^{18}F–choline PET/CT could introduce an amazing improvement in the management of prostate cancer patients. In fact, the value of ^{18}F–choline uptake could provide to clinicians prognostic and predictive information about the growth of the analyzed cancers. From biological point of view, the association between ^{18}F–choline uptake and Ki67 can be explained by the role this molecule plays in the cell membrane formation [34].

According to the conflicting data reported in the literature, no association was found between the value of free PSA serum concentration and ^{18}F–choline uptake. Specifically, we observed some patients with very low value of PSA serum concentration and high uptake of ^{18}F–choline. In addition, histological analysis displayed a GG of 4+3 indicating the presence of “intermediate risk cancer”. Nevertheless, large cohort population studies are needed to clarify these data.

Conclusion

The identification of in vivo analysis capable to provide prognostic and predictive data on prostate cancer lesions represents one of the most important challenges of the scientific community. In this pilot study, for the first time, we propose the use of ^{18}F–choline PET/CT to identify undifferentiated prostate cancer lesions characterized by high proliferation index. These data, if confirmed on large population, can be used to improve the current clinical practices planned for the management of prostate cancer patients.

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Conflicts of Interest

There are no potential conflicts of interest relating to the manuscript (for each author). The study is original and the manuscript has not been published yet and is not being considered for publication. All authors have agreed with the submission in its present (and subsequent) forms.

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