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Abbreviations: AS, active surveillance; GG1, Gleason Grade Group 1; IQR, interquartile range; PCa, prostate cancer; RP, radical prostatectomy.

The clinical characteristics of patients with primary non-prostate-specific membrane antigen-expressing prostate cancer on preoperative positron emission tomography/computed tomography

Prostate-specific membrane antigen (PSMA) positron emission tomography (PET)/CT is a modern imaging tool used in the diagnosis of prostate cancer (PCa). About 10% of patients who undergo PSMA PET/CT have a biopsy confirmed, primary PCa without PSMA expression on PET/CT (non-$PSMA_{PET}$-expressing PCa) according to a recent systematic review [1]. However, the definition of non-$PSMA_{PET}$-expression in these studies was poorly defined and no immunohistochemical studies for confirmation of PSMA protein expression were performed. The aim of this retrospective study was to report the prevalence, characteristics, and immunohistochemical assessment of non-$PSMA_{PET}$-expressing hormone-sensitive PCa in a cohort of 362 patients who underwent PSMA PET/CT and robot-assisted radical prostatectomy (RARP).

This study was approved by the local Institutional Review Board (IRBdm19-348). All patients had biopsy confirmed, hormone-sensitive, D'Amico intermediate- to high-risk PCa and were surgically treated in a high-volume RARP centre between 2016 and 2020. The patients underwent PSMA PET/CT with the radiotracers ^{68}Ga-PSMA-11, ^{18}F-DCFPyL or ^{18}F-PSMA-1007. PET images from skull to mid-thigh were performed at a median (interquartile range [IQR]) of 49 (45–56) min post-injection after a median (IQR) bolus injection of 101 (93–110) MBq fixed-dose for ^{68}Ga-PSMA-11, 73 (57–83) min post-injection after a median (IQR) injected dose of 239 (202–299) MBq for ^{18}F-DCFPyL, and 80 (74–92) min post-injection after a median (IQR) dose of 281 (252–304) MBq for ^{18}F-PSMA-1007. All PSMA PET/CTs were centrally reviewed by experienced nuclear medicine specialists. Non-$PSMA_{PET}$-expressing PCa was defined as no focal tracer uptake in the prostate exceeding the background activity of normal prostate tissue, in accordance with Prostate Cancer Molecular Imaging Standardised Evaluation (PROMISE) criteria and the European Association of Nuclear Medicine PSMA (E-PSMA) guidelines [2]. All patients underwent a bi- or multiparametric MRI at 1.5 or 3 Tesla. All MRI scans were centrally reviewed by experienced uroradiologists. Clinical, radiological, and histopathological data were collected from the prospectively maintained institutional database. Immunohistochemical staining was performed on all non-$PSMA_{PET}$-expressing tumours and a 2:1 matched group of $PSMA_{PET}$-expressing tumours. Matching was based on radiotracer, pathological tumour stage, and Gleason score. The staining was performed with an anti-PSMA monoclonal mouse antibody (Clone 3E6; DAKO, North America Inc., Carpinteria, CA, USA). One uropathologist (E.B.) assessed the tumour area in a 20 mm^2 region of interest in the slide and the proportion of tumour with commonly used different staining intensities: no expression, weak expression, moderate expression, intense expression. The overall staining intensity was calculated with the histoscore (H-score), ranging from 0 to 300 [3]. Differences in non-$PSMA_{PET}$-expressing PCa and $PSMA_{PET}$-expressing PCa regarding clinical, radiological, pathological and immunohistochemical data were analysed with Pearson chi-square test, Fisher's exact test, Student's *t*-test, or Mann–Whitney *U*-test. Statistical significance was set on $P = 0.05$.

The prevalence of non-$PSMA_{PET}$-expressing PCa was 4.1% (15/362). Comparable prevalence was reported for the different radiotracers: ^{68}Ga-PSMA-11: 3.5% (eight of 228); ^{18}F-DCFPyL: 5.4% (six of 112); ^{18}F-PSMA-1007: 4.5% (one of

Table 1 Preoperative characteristics of 362 patients with non-$PSMA_{PET}$-expressing and $PSMA_{PET}$-expressing PCa on PSMA PET/CT.

Characteristic	Non-$PSMA_{PET}$-expressing	$PSMA_{PET}$-expressing	*P*
Patients, *n* (%)	15 (4.1)	347 (96)	
PSA level, ng/L, median (IQR)	6.8 (4.1–9.4)	11 (7.5–21)	0.001
Clinical tumour stage, *n* (%)			
cT1c	4 (27)	121 (35)	0.81
cT2a–c	8 (53)	166 (48)	
cT3a–b	3 (20)	60 (17)	
Biopsy Gleason scores, *n* (%)			
3 + 3	0 (0)	2 (7.8)	0.43
3 + 4	4 (27)	61 (18)	
4 + 3	4 (27)	76 (22)	
3 + 5; 4 + 4; 5 + 3	6 (40)	108 (31)	
4 + 5; 5 + 4; 5 + 5	1 (6.7)	74 (21)	
D'Amico high-risk, *n* (%)	14 (93)	318 (92)	0.99
Suspect nodes on PSMA PET/CT; miN1, *n* (%)	2 (13)	46 (13)	0.99
Distant metastases on PSMA PET/CT; miM1, *n* (%)	0 (0)	12 (3.5)	0.99
Briganti score, %, median (IQR)	17 (11–36)	19 (9.3–42)	0.80
MRI tumour volume, mL, median (IQR)	2.0 (0.7–4.2)	2.5 (0.9–7.0)	0.47
ADC value, median (IQR)	858 (809–1024)	838 (726–1000)	0.33
PI-RADS category, *n* (%)			
No visual lesion	1 (6.7)	8 (2.1)	0.71
3	0 (0)	15 (4.3)	
4	3 (20)	56 (22)	
5	11 (73)	258 (74)	
Pathological tumour stage, *n* (%)			
pT0-Tx	0 (0)	2 (0.6)	1.0
pT2a–c	6 (40)	14 (43)	
pT3a–b	9 (60)	197 (57)	
Pathological Gleason score, *n* (%)			
3 + 3	1 (6.7)	9 (2.6)	0.57
3 + 4	6 (40)	127 (37)	
4 + 3	3 (20)	116 (33)	
3 + 5; 4 + 4; 5 + 3	1 (7)	32 (9.2)	
4 + 5; 5 + 4; 5 + 5	4 (27)	59 (17)	
Unknown*	0 (0)	4 (1.2)	
Pathological nodal stage, *n* (%)			
pN0	13 (88)	220 (63)	0.13
pN1	1 (6.7)	81 (23)	
pNx	1 (6.7)	46 (13)	
Positive surgical margin status (R1), *n* (%)	4 (27)	137 (40)	0.42
Intraductal growth present, *n* (%)	4 (27)	74 (21)	0.74
Cribriform growth present, *n* (%)	10 (67)	223 (64)	0.82

*ADC, apparent diffusion coefficient; R, surgical margin status. *The Gleason score of the prostatectomy specimen could not be assessed due to neoadjuvant hormonal therapy use.*

22). The initial serum PSA level was significantly lower in patients with non-$PSMA_{PET}$-expressing PCa than in those with $PSMA_{PET}$-expressing PCa, at a median (IQR) of 6.8 (4.1–9.4) vs 11 (7.5–21) ng/L ($P = 0.001$, Table 1). There were no differences between both cohorts for clinical tumour stage, biopsy Gleason score, molecular imaging nodal (miN1) or distant metastasis stage (miM1), or D'Amico high-risk PCa. Also, there were no differences in Prostate Imaging Reporting and Data System (PI-RADS) scores or MRI tumour volumes (median 2.0 vs 2.5 mL, $P = 0.47$) [4].

There were no differences between the cohorts for pathological tumour stage, RARP specimen Gleason score, positive surgical margin rate, presence of intraductal growth or cribriform growth patterns. In patients who underwent an extended pelvic lymph node dissection, lymph node metastases (pN1) were found in one of 14 (7%) patients with non-$PSMA_{PET}$-expressing PCa vs 81/301 (27%) patients with $PSMA_{PET}$-expressing PCa ($P = 0.13$).

None of the investigated tumours, including non-$PSMA_{PET}$-expressing tumours, fully lacked immunohistochemical PSMA protein expression. Non-$PSMA_{PET}$-expressing tumours had a significantly smaller proportion of tumour cells with either moderate or intense PSMA protein expression compared to $PSMA_{PET}$-expressing tumours, at a median (IQR) of 35 (15–70)% vs 85 (70–96)% ($P = 0.001$). The overall staining intensity (H-score) was significantly lower in non-$PSMA_{PET}$-expressing tumours compared to $PSMA_{PET}$-expressing tumours, at a median (IQR) of 135 (90–170) vs 210 (170–285) ($P = 0.001$).

This study is the first to show the characteristics of patients with non-$PSMA_{PET}$-expressing PCa and the first to report on the immunohistochemical analysis of these tumours. With a

definition that adheres to recently proposed guidelines, the prevalence of non-$PSMA_{PET}$-expressing PCa in the present study was lower (4.1%) than the previously reported 10% [1]. This is consistent with more recent studies evaluating local tumour presence on PSMA PET/CT that reported a 4.3–5.9% prevalence [5,6]. Previous authors tried to determine a cut-off value for $PSMA_{PET}$-expressing PCa based on the maximum standard uptake value (SUV_{max}). However, SUV_{max} measurement is influenced by a number of tracer-related and technical factors (e.g. dosage, injection time, voxel size, number of iterative updates of ordered subset expectation maximisation). Due to the multicentre aspect of this study, technical inconsistencies were present, probably leading to unreliable SUV_{max} measurements. Therefore, SUV_{max} was omitted from the analysis. We showed that, except for a lower serum PSA level, non-$PSMA_{PET}$-expressing PCa and $PSMA_{PET}$-expressing PCa have no different clinical, radiological, and histopathological characteristics. Moreover, as the majority of non-$PSMA_{PET}$-expressing tumours proved to be (plain) adenocarcinoma after histopathological examination, it is to be doubted that these tumours represent de-differentiated, neuroendocrine PCa [7,8]. In fact, non-$PSMA_{PET}$-expressing tumours are presumably hormone-sensitive PCas that have similar preoperative and histopathological features compared to $PSMA_{PET}$-expressing PCas. It needs to be acknowledged that the aetiology of non-$PSMA_{PET}$ expression is probably multifactorial and not just a consequence of dismal prognostic tumour features. We showed that non-$PSMA_{PET}$ expression is associated with decreased immunohistochemical PSMA protein expression when compared to $PSMA_{PET}$-expressing PCa. The proportion of cells with moderate and intense immunohistochemical PSMA protein expression was significantly lower in the non-$PSMA_{PET}$-expressing tumours compared to the $PSMA_{PET}$-expressing tumours, as well as the overall PSMA-staining intensity (H-score). However, none of the non-$PSMA_{PET}$-expressing tumours fully lacked immunohistochemical PSMA protein expression. Therefore, it is likely that technical factors with respect to PSMA PET/CT imaging itself or due to the biodistribution of the radiotracer may have partially contributed to the phenomenon of non-$PSMA_{PET}$ expression.

This study is limited by the retrospective nature of the cohort, the different radiotracers and the different scanning protocols used. However, to reduce bias, all scans were centrally reviewed, and all non-$PSMA_{PET}$-expressing scans were re-reviewed by an experienced nuclear medicine specialist (M.D.) who had access to pre- and postoperative data. Also, no difference in the prevalence of non-$PSMA_{PET}$-expressing PCa was found when comparing the different radiotracers. Due to the low prevalence of non-$PSMA_{PET}$ expression, the group size was small, which limits the statistical power of the analyses.

The detection of lymph node metastases with PSMA PET/CT may be unreliable in those with non-$PSMA_{PET}$-expressing PCa. Clinicians could consider performing a bone scan in patients with a high a priori risk of distant metastases (PSA level of >20 ng/L or Gleason score 9–10) in the case of non-$PSMA_{PET}$-expressing PCa to determine the eligibility of curative treatment. Future studies should focus on the intermediate- to long-term oncological outcomes of patients with non-$PSMA_{PET}$-expressing PCa.

In conclusion, the prevalence of non-$PSMA_{PET}$-expressing PCa in a large contemporary cohort was low. Apart from a lower serum PSA level, patients with non-$PSMA_{PET}$-expressing tumours had similar clinical, radiological, and histopathological features to those with $PSMA_{PET}$-expressing tumours. Non-$PSMA_{PET}$-expressing tumour cells had significantly lower immunohistochemical PSMA protein expression than $PSMA_{PET}$-expressing tumour cells.

Disclosure of Interest

None declared.

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Abbreviations: IQR, interquartile range; miM, molecular imaging distant metastases as assessed on PSMA PET/CT; miN, molecular imaging nodal stage as assessed on PSMA PET/CT; PCa, prostate cancer; PET, positron emission tomography; PI-RADS, Prostate Imaging Reporting and Data System; pN, pathological nodal stage; PSMA, prostate-specific membrane antigen; RARP, robot-assisted radical prostatectomy; SUV_{max}, maximum standard uptake value.