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SHORT COMMUNICATION

Low hexokinase-2 expression-associated false-negative ^{18}F-FDG PET/CT as a potential prognostic predictor in patients with multiple myeloma

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Abstract

Purpose False-negative ^{18}F-FDG PET/CT, which is associated with low hexokinase-2 (HK2) expression in multiple myeloma (MM), is a new concept that is relevant for diagnosis and treatment response assessment. This study aimed to investigate the prognostic relevance of low HK2 expression-associated false-negative PET/CT in patients with MM.

Methods Ninety consecutive patients, with newly diagnosed MM, receiving novel agents during induction therapy were enrolled in this retrospective study. Patients were divided into three groups according to the combination of the positivity of PET/CT and whole-body diffusion-weighted magnetic resonance imaging (DWMRI), namely, negative DWMRI, false-negative PET/CT, and positive PET/CT.

Results False-negative PET/CT was observed in 12% patients who were older, had documented clinical history of smouldering MM, and showed lower HK2 expression levels than the positive PET/CT patients. False-negative PET/CT patients showed a clear trend of longer time to next treatment (TTNT) and progression-free survival (PFS) than the positive PET/CT patients ($P = 0.035$ and 0.071, respectively). Furthermore, TTNT and PFS of false-negative PET/CT patients were similar to those of patients without established high-risk PET/CT findings and significantly longer than those of high-risk PET/CT patients ($P = 0.013$ and 0.047, respectively).

Conclusions This study showed, for the first time, that low HK2 expression-associated false-negative PET/CT was associated with relatively better prognosis in patients with newly diagnosed MM, suggesting that this phenomenon may not undermine the established PET/CT-based prognostication. Furthermore, this phenomenon may be useful for identifying patients at lower risk of disease progression among those with myelomatous lesions on DWMRI.

Keywords False-negative · Hexokinase-2 · Multiple myeloma · Positron emission tomography/computed tomography · Prognosis

Introduction

False-negativity of ^{18}fluorine-fluoro-deoxyglucose (^{18}F-FDG) positron emission tomography/computed tomography (PET/CT) is an emerging idea in the field of multiple myeloma (MM) diagnosis, which was introduced by Rasche et al. in 2017 [1]. This is defined by the presence of myelomatous diseases that can be detected using whole-body diffusion-weighted magnetic resonance imaging (DWMRI) but not by PET/CT. The incidence of this phenomenon is approximately 11%, and further investigations revealed that it was strongly associated with low hexokinase-2 (HK2) expression of myeloma, which is the enzyme catalysing the rate-limiting and first obligatory step of the glycolytic pathway [1]. This potential clinical limitation of PET/CT is of particular interest, as PET/CT assessment is recommended for the diagnosis and evaluation of treatment

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response by the International Myeloma Working Group (IMWG) [2, 3]. Furthermore, PET/CT is now considered to be a valuable tool in the prognostic assessment of MM [3, 4]. Therefore, the influence of PET/CT false-negativity on prognostic assessment requires further elucidation.

HK2 has emerged as a potential prognostic marker of solid cancers [5]. Several meta-analyses have demonstrated that low HK2 expression of tumour cells was associated with better prognosis in various types of solid cancers, especially in digestive system cancers [6, 7]. However, these associations have not been explored in haematological malignancies so far. Hence, the aim of this study was to investigate the prognostic relevance of low HK2 expression-associated false-negative PET/CT in patients with newly diagnosed MM.

Methods

We retrospectively identified 92 consecutive patients with newly diagnosed symptomatic MM who were diagnosed and treated between 2015 and 2018 at the Kameda Medical Centre, Kamogawa, Japan. MM diagnosis and treatment response were assessed using the IMWG criteria [2, 8]. All patients underwent pretreatment ^{18}F-FDG PET/CT assessment concurrently with whole-body DWMRI imaging with the time interval of within 1 week. PET/CT was performed using a PET/CT scanner (Discovery ST Elite Performance; GE Healthcare, Milwaukee, USA). DWMRI imaging was performed on a 1.5 Tesla unit (Magnetom Vision; Siemens Healthcare, Erlangen, Germany). To maximise homogeneity of chemotherapy regimens and improve the prognostic value of our analyses, we excluded patients who had been treated without novel agents (e.g., immunomodulatory agents or proteasome inhibitors) during induction therapies ($n = 2$). Ultimately, 90 patients were included in our analyses. All participants had provided written comprehensive informed consent not only for undergoing PET/CT and DWMRI, but also for secondary use of their bone marrow (BM) samples and clinical data in the future, including PET/CT and DWMRI findings and patient outcomes for retrospective studies. The study was conducted according to the Declaration of Helsinki and was approved by the review board of the Kameda Medical Centre.

The positivity of a focal lesion on PET/CT and DWMRI images was defined as previously described (details in Supplemental Methods) [1]. Patients were divided into three groups according to the combination of the positivity of PET/CT and DWMRI (PET-DWMRI category) [1]: negative DWMRI (no detectable disease by either method), false-negative PET/CT (disease detectable by DWMRI only), and positive PET/CT (disease detectable by both methods). None of the patients were DWMRI-negative but PET/CT-positive (Fig. 1). High-risk PET/CT findings in this study were defined as the presence of >3 focal lesions as established previously [3]. HK2 expression levels were analysed using real-time reverse transcription polymerase chain reaction in CD138-enriched plasma cells using BM samples of patients with positive or false-negative PET/CT findings (details in Supplemental Methods). For comparison, HK1, HK3, HK4, and solute carrier family 2, member 1 (SLC2A1) expression levels were also measured using the same methods.

The relationship between baseline characteristics and PET/CT findings in combination with DWMRI findings was compared using one-way analysis of variance, the Kruskal–Wallis test, or chi-squared test as appropriate. To focus on the disease factors of myeloma, the primary end point of this study was set as time to next treatment (TTNT) and progression-free survival (PFS). The probability of TTNT, PFS, and overall survival (OS) was estimated using the Kaplan–Meier method and was compared using the log-rank test. A two-tailed P value <0.05 was considered statistically significant. Statistical analysis was performed using the R (v3.1.2) software.

Results

Baseline clinical characteristics of all patients are summarised in Table 1. The median age of the patients was 74 (IQR: 67–81) years. The median observation period of all patients was

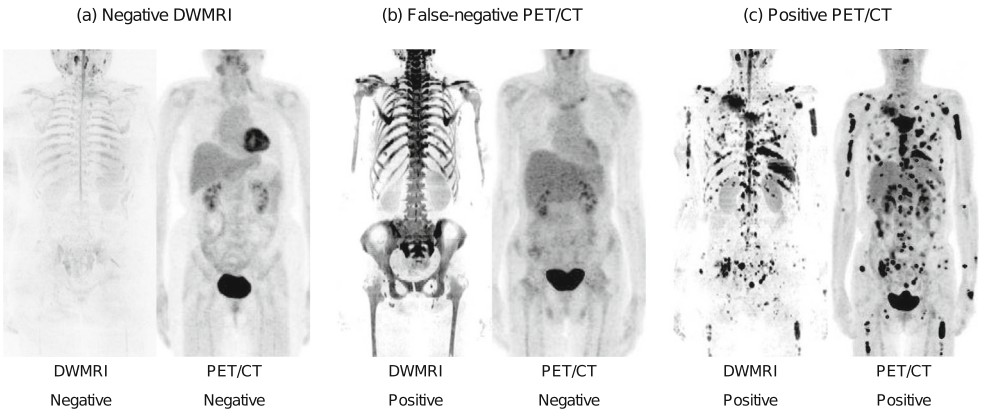

Fig. 1 Categorisation of patients according to the combined observations of PET/CT and whole-body diffusion-weighted magnetic resonance imaging (DWMRI). (**a**) Negative DWMRI (no detectable disease by either method, (**b**) false-negative PET/CT (disease detectable by DWMRI only) and, (**c**) positive PET/CT (disease detectable by both methods)

Table 1 Comparison of clinical characteristics of patients according to the categories classified per the combination of PET/CT and DWMRI findings

Clinical factors	All cohort	PET-DWMRI category†			*P* value
	N = 90	Negative DWMRI (−/−) N = 21 (23.3%)	False-negative PET/CT (−/+) N = 11 (12.2%)	Positive PET/CT (+/+) N = 58 (64.4%)	
Age, years [median (IQR)]	74 (67, 81)	69 (64, 78)	80 (78, 86)	73 (67, 81)	0.001
Sex, male (%)	40 (44.4)	11 (52.4)	4 (36.4)	25 (43.1)	0.64
Clinical history of smouldering myeloma (%)	7 (7.8)	4 (19.0)	3 (27.3)	0 (0.0)	0.001
Heavy chain type, IgG (%)	48 (53.3)	14 (66.7)	4 (36.4)	30 (51.7)	0.24
Albumin, g/dL [median (IQR)]	3.3 (2.6, 3.9)	3.5 (2.6, 3.8)	3.3 (2.8, 3.7)	3.2 (2.6, 3.9)	0.98
Beta 2-microglobulin, mg/L [median (IQR)]	5.2 (3.1, 9.1)	3.3 (2.4, 6.9)	4.5 (3.6, 8.1)	5.9 (3.4, 11.3)	0.16
Creatinine, mg/dL [median (IQR)]	0.98 (0.72, 1.61)	0.96 (0.60, 2.55)	0.95 (0.71, 1.14)	1.02 (0.78, 1.53)	0.77
Haemoglobin, g/dL [median (IQR)]	9.9 (8.4, 11.8)	10.2 (9.6, 11.7)	9.4 (7.9, 10.0)	9.7 (8.3, 12.2)	0.15
LDH, high (%)	32 (35.6)	7 (33.3)	4 (36.4)	21 (36.2)	0.97
High-risk CA (%)	23 (25.6)	3 (14.3)	3 (27.3)	17 (29.3)	0.39
Del(17p)	10 (11.1)	1 (4.8)	1 (9.1)	8 (13.8)	0.51
t(4;14)	10 (11.1)	2 (9.5)	2 (18.2)	6 (10.3)	0.72
t(14;16)	3 (3.3)	0 (0.0)	0 (0.0)	3 (5.2)	0.43
ISS, stage III (%)	48 (53.3)	7 (33.3)	6 (54.5)	35 (60.3)	0.10
R-ISS, stage III (%)	27 (30.0)	3 (14.3)	4 (36.4)	20 (34.5)	0.20
Bone marrow PCs, % [median (IQR)]	15.1 (6.1, 34.0)	10.2 (6.1, 16.9)	13.0 (3.5, 27.8)	20.8 (6.9, 41.5)	0.11
Induction regimen (%)					
Doublet	19 (21.1)	3 (14.3)	4 (36.4)	12 (20.7)	0.35
Triplet	71 (78.9)	18 (85.7)	7 (63.6)	46 (79.3)	
Post-induction ASCT recipients (%)	33 (36.7)	10 (47.6)	1 (9.1)	22 (37.9)	0.094
Best response, CR or better (%)	42 (46.7)	9 (42.9)	4 (36.4)	29 (50.0)	0.65
Maintenance after CR or better (%)††	36 (85.7)	8 (88.9)	3 (75.0)	25 (86.2)	0.79

Abbreviations: ASCT, autologous stem cell transplantation; CA, cytogenetic abnormality; CR, complete response; DWMRI; diffusion-weighted magnetic resonance imaging, IQR, interquartile range; ISS, International Staging System; LDH, lactate dehydrogenase; PCs, plasma cells; PET/CT, positron emission tomography/computed tomography; R-ISS, revised International Staging System

† Patients were divided into three groups according to the combination of the positivity of PET/CT and DWMRI: negative DWMRI (no detectable disease by either method), false-negative PET/CT (disease detectable by DWMRI only), and positive PET/CT (disease detectable by both methods)

†† N = 42 (negative DWMRI, *n* = 9; false-negative PET/CT, *n* = 4; positive PET/CT, *n* = 29)

23.1 (IQR: 15.2–30.0) months. The majority of patients (78.9%) were treated with triplet induction regimens.

There were 21 (23.3%), 11 (12.2%), and 58 (64.4%) patients with negative DWMRI, false-negative PET/CT, and positive PET/CT findings, respectively. The comparison of clinical characteristics across the three groups has been summarised in Table 1. Notably, false-negative PET/CT patients were significantly older and had a documented clinical history of smouldering (i.e., asymptomatic) MM than other patients. Positive PET/CT patients tended to harbour high-risk cytogenetic abnormalities and higher disease stage, although the differences were not statistically significant, whereas no inter-group difference was detected across the groups regarding other myeloma-related parameters, induction regimens, and treatment responses. As previously demonstrated, false-negative PET/CT patients showed significantly lower expression levels of HK2 than positive PET/CT patients ($P < 0.001$) (Fig. 2), whereas no inter-group difference was detected in HK1, HK3, and SLC2A1 expression levels (data not shown); significant HK4 expression was not detected in either group.

The Kaplan–Meier survival curves of TTNT and PFS according to the PET-DWMRI categories are shown in Fig. 3.

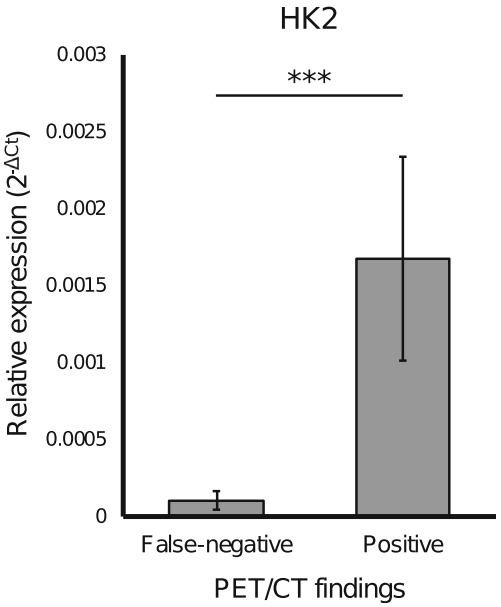

Fig. 2 Comparison of hexokinase-2 (HK2) expression levels of myeloma between false-negative and positive PET/CT patients. Real-time reverse transcription polymerase chain reaction analysis of HK2 expression in bone marrow CD138-positive cells. *Bars* indicate the mean ± standard error. Asterisks (*) indicate statistical significance: ***$P < 0.001$. The Student t-test was used to examine statistical significance

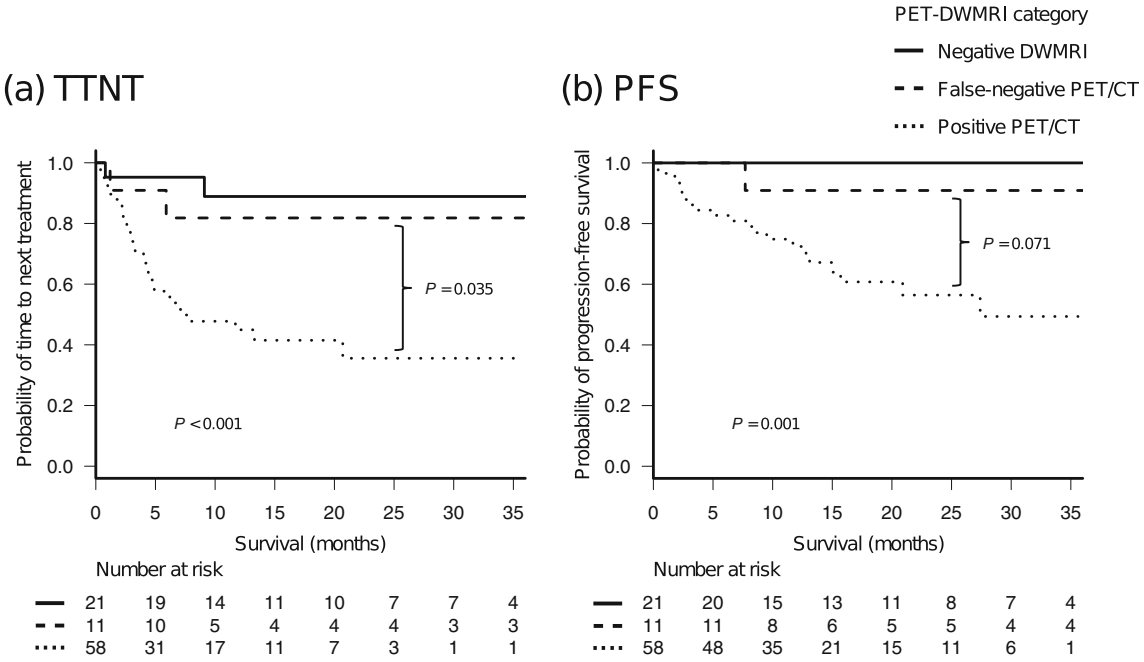

Fig. 3 Treatment outcomes based on the combined findings of PET/CT and whole-body diffusion-weighted magnetic resonance imaging (DWMRI) (PET-DWMRI category). (**a**) Time to next treatment (TTNT) and (**b**) progression-free survival (PFS) of patients with negative DWMRI, false-negative PET/CT, and positive PET/CT findings

Negative DWMRI patients showed excellent TTNT and PFS, whereas positive PET/CT patients showed shorter TTNT and PFS than other patients ($P < 0.001$ and 0.001 for TTNT and PFS, respectively). Among the 69 patients with positive DWMRI findings (false-negative and positive PET/CT patients), false-negative PET/CT patients showed a clear trend of longer TTNT and PFS, although the difference in PFS was not statistically significant ($P = 0.035$ and 0.071 for TTNT and PFS, respectively). This trend was also observed in the OS analysis (Fig. S1). We further investigated the prognostic relevance of false-negative PET/CT in the context of the established high-risk PET/CT findings. Notably, the TTNT,

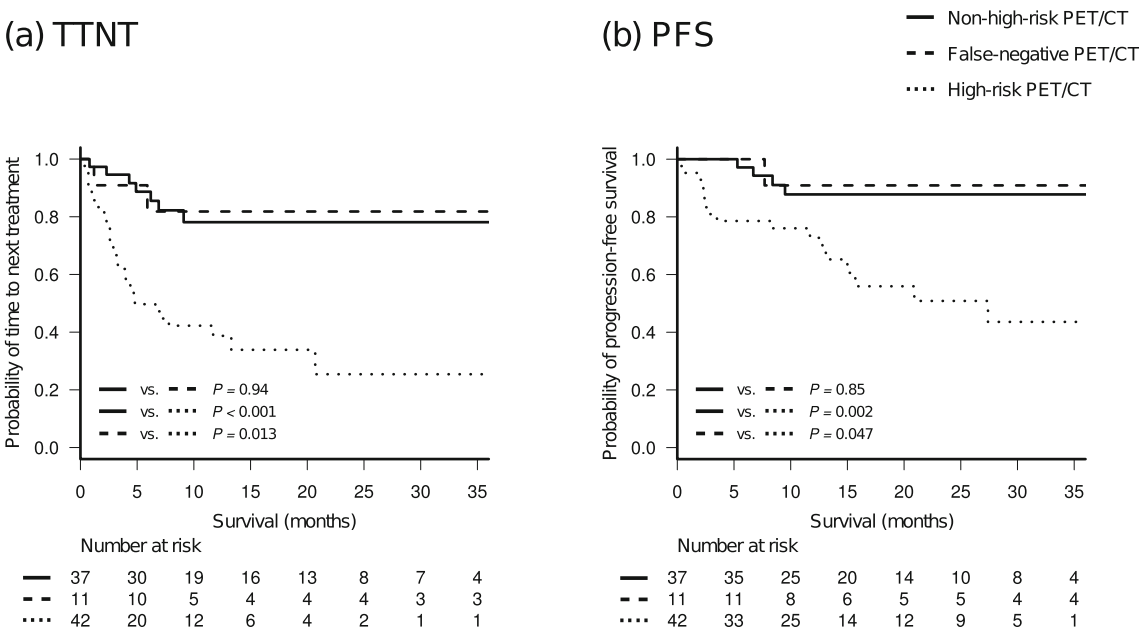

Fig. 4 Comparison of treatment outcomes between false-negative PET/CT patients and patients with or without established high-risk PET/CT findings. (**a**) Time to next treatment (TTNT) and (**b**) progression-free survival (PFS) of false-negative PET/CT patients and the remaining patients with or without established high-risk PET/CT findings (i.e., the presence of more than three focal lesions on PET/CT)

PFS, and OS curves of false-negative PET/CT patients were extremely close to those of non-high-risk PET/CT patients: both TTNT and PFS in false-negative PET/CT patients were significantly longer than those in high-risk PET/CT patients ($P = 0.013$ and 0.047 for TTNT and PFS, respectively; Figs. 4 and S2).

Discussion

In the present study, we validated the incidence of false-negative PET/CT and its association with low expression of HK2. Furthermore, we observed that false-negative PET/CT patients tended to show better prognosis than PET/CT-positive patients. Their TTNT and PFS were similar to those of negative or non-high-risk PET/CT patients and were significantly longer than those of high-risk PET/CT patients. These results are of particular importance because these indicate that false-negative PET/CT phenomenon might have had only minor influence on the results in previous studies that have investigated the prognostic significance of PET/CT findings; false-negative PET/CT patients may be included in the group of non-high-risk PET/CT patients in prognostic analyses. In this respect, our results might underscore the established prognostic performance of PET/CT in MM [3]. Furthermore, false-negative PET/CT findings may be useful for distinguishing between prognoses for patients with positive myelomatous disease on DWMRI.

Intriguingly, false-negative PET/CT patients were older than other patients, which might be associated with the high proportion of patients with a documented clinical history of smouldering MM among them [9]. These findings suggested that false-negative PET/CT patients tended to present more indolent clinical courses. Furthermore, the fact that patients with false-negative PET/CT findings showed better prognosis despite the advanced age as well as the absence of inter-group differences in treatment might emphasize the favourable prognostic impact of this phenomenon. Indeed, only one patient (9.1%) among false-negative PET/CT patients underwent autologous stem cell transplantation.

As previously described [1], low HK2 expression levels were observed in false-negative PET/CT patients. Previous studies have suggested that HK2 expression levels were associated with tumour growth and prognosis in various solid tumours [10]. Our results are in agreement with these previous observations, indicating that similar mechanisms might be functional in MM. Therefore, further studies are required to investigate the prognostic relevance of HK2 expression levels per se in MM.

Although this study had several limitations, including its retrospective nature, small sample size, and relatively short observation period, we believe that it provides unique clinical and biological perspectives for research in the field of MM.

In conclusion, we for the first time, showed that low HK2 expression-associated false-negative ^{18}F-FDG PET/CT was associated with relatively better prognosis in patients with newly diagnosed MM. These results suggest that this phenomenon may facilitate the identification of patients at lower risk of disease progression among those with myelomatous lesions on DWMRI, and that it might not interfere with the results of previous as well as future studies that investigate the prognostic performance of PET/CT. Further studies are required to validate and expand our findings.

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Author contributions YA, AK, and KM conceived and designed the study. YA collected data, performed statistical analyses, and wrote the manuscript. SI performed experiments for quantification of HK2 expression. YA, AK, KN, HK, DM, MT, and KM provided patient care. EO and TO interpreted the PET/CT images. KM supervised the study. All authors reviewed and approved the manuscript.

Compliance with ethical standards

Conflict of interest statement The authors declare that they have no conflict of interest.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent All participants provided written comprehensive informed consent both for undergoing PET/CT and DWMRI, and also for secondary use of their bone marrow (BM) samples and clinical data in the future, including PET/CT and DWMRI findings and patient outcomes for retrospective studies.

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