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Whole Skeletal Mean SUV Measured on ^{18}F-NaF PET/CT Studies as a Prognostic Indicator in Patients with Bone Metastatic Breast Cancer.

Short title: ^{18}F-NaF and breast cancer prognosis

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Abstract

In this work we assessed the association between the whole skeletal mean standardized uptake value (SUV) measured on ^{18}F-NaF PET/CT studies and the overall survival (OS) of bone metastatic breast cancer patients. **Methods:** We retrospectively analyzed 176 patients with breast cancer and bone metastatic disease who performed ^{18}F-NaF PET/CT studies. The outcomes of the patients (dead or alive) were established based on the last information available on their files. The mean and maximum SUVs were measured in a whole skeletal volume of interest (wsVOI). The wsVOI was defined based on the CT component of the PET/CT study using Hounsfield Units thresholds. The wsVOI was then applied on the ^{18}F-NaF PET image. Univariate analyses were performed to assess the association of the SUVs with OS. We also analyzed the association of the age of the patients, the presence of visceral metastatic disease, histological subtypes, presence of hormone receptors, human epidermal growth factor receptor 2 expression and the creatinine, CA15-3 and alkaline phosphatase (ALP) levels with OS. The variables statistically significant in the univariate analyses were included in a multivariate cox regression survival analysis. **Results:** In the univariate analyses there were associations of the mean and maximum whole skeletal SUVs, estrogen receptor status and the CA15-3 and ALP levels with OS. In the multivariate analysis, all the variables that were statistically significant in the univariate analysis but the CA15-3 were associated with OS. **Conclusions:** In patients with bone metastatic breast cancer, the whole skeletal mean SUV is an independent predictor of overall survival.

Key Words: ^{18}F; PET/CT; ^{18}F-NaF; breast cancer; bone metastases.

Introduction

^{18}F-NaF PET/CT has been used for the detection of bone metastases in neoplastic diseases, mainly in those with osteoblastic metastases (*1*,*2*). It has higher accuracy than ^{99m}Tc-labelled diphosphonate bone scan even when SPECT or SPECT/CT techniques are utilized (*3*–*5*) and this higher accuracy associated with the growing availability of PET/CT equipment (*6*,*7*) are resulting in the increasing use of ^{18}F-NaF PET/CT in the clinical assessment of bone metastatic disease (*8*,*9*).

Beside the detection of skeletal metastases, some articles have described the capability of the ^{18}F-NaF PET/CT to measure the degree of bone metastatic disease (*10*,*11*) and the utility of this measurement in the follow up (*12*) and in the estimation of the overall survival in patients with prostate cancer (*13*) and, less frequently, with other neoplastic disease as breast cancer (*14*), medullary thyroid cancer (*15*), multiple myeloma (*16*) and urological malignancies (*17*). Thus, at present, the utility of ^{18}F-NaF PET/CT in cancer patients is not restricted to the detection of metastatic bone disease but could also be extended to the estimation of the burden of skeletal disease since this estimation is useful in the follow up of the patients and allows prognostic assessment.

However, the methodology used to estimate the burden of metastatic disease is a work in progress and there is not yet a definitive method for this measurement.

Therefore, in the presented article we described a methodology to calculate the whole skeletal mean standardized uptake value (SUV) in ^{18}F-NaF PET/CT studies and also analyzed this parameter as an indicator of the burden of bone metastatic disease and its independent association with the overall survival in patients with breast cancer.

Material and Methods

Patients

We retrospectively analyzed 176 female patients with bone metastatic breast cancer who underwent ^{18}F-NaF PET/CT studies in our institution between 2011 and 2018 and that performed creatinine, CA15-3 and alkaline phosphatase analyses within 3 months of the PET/CT studies. This group represents all patients found in the files with the aforementioned characteristics. The information on age of the patients, the results of the ^{18}F-NaF PET/CT, the presence of visceral metastases, the creatinine, CA15-3 and alkaline phosphatase levels, histological subtypes, hormone receptors status, human epidermal growth factor receptor 2 (HER-2) expression, time from diagnosis to the ^{18}F-NaF PET/CT studies, and the patients' survival were also obtained from the electronic medical record files.

The age of the patients were measured at the time of the ^{18}F-NaF PET/CT studies. The outcomes of the patients (dead or alive) were established based on the last information available on the electronic files and the information on the presence of soft tissue metastases was obtained based on the results of other imaging studies available at the moment of the ^{18}F-NaF PET/CT studies.

The approval by the local Research and Ethics Committee was obtained before this retrospective study was initiated and the requirement to obtain informed consent was waived.

^{18}F-NaF PET/CT studies

The ^{18}F-NaF PET/CT images were acquired around 45 to 60 min after injection of approximately 185 MBq of the radiopharmaceutical on a Discovery 690® PET/CT scanner with Time of Flight (General Electric, Waukesha, Wisconsin, USA). All patients underwent whole-

body (vertex to toes) 3-dimensional PET/CT acquisition. The emission images were obtained at 1 min per bed position (15 cm axial scan field of view with 3 cm of overlapping), with 13 to 15 bed positions per study. The CT transmission images (30 mAs) were obtained for attenuation correction and anatomic correlation of the uptake areas. Other CT acquisition parameters were: 120 kVp, 0.5-s rotation time, 1.375 pitch and axial slice thickness of 3.75 mm. The PET image reconstruction was performed using iterative technique (OSEM) with 2 iterations and 24 subsets for all studies. CT images reconstruction was based on conventional filtered back projection (FBP) method with GE BONE PLUS filter.

Determination of the presence of skeletal metastases

The definition of the presence of bone metastatic disease was based on findings of the ^{18}F-NaF PET/CT studies (metabolic and anatomical patterns), on the results of other imaging studies when available (MRI and high-resolution contrast enhanced CT images), and on the follow up studies performed either with ^{18}F-NaF PET/CT or ^{99m}Tc-MDP. Patients without bone metastases were excluded from the study.

Calculation of whole skeletal SUVs

The degree of the ^{18}F-NaF uptake in the skeletal was measured in a whole skeletal volume of interest (wsVOI) using the maximum SUV (SUVmax) and the mean SUV (SUVmean) within the wsVOI. The wsVOI was defined based on the CT component of the PET/CT study using a Hounsfield unit (HU) threshold (usually 120 HU). The wsVOI was then applied on the metabolic image of the PET/CT study (Figures 1, 2 and 3). This was done using AMIDE® software (*18*).

Statistical analyses

The statistical analyses were divided in two phases. First, univariate analyses were performed to assess the association of the variables: whole skeletal SUVmean and SUVmax, age, presence of visceral metastases, histological subtypes (invasive ductal and invasive lobular carcinomas), presence of hormone receptors (estrogen and progesterone), HER2 expression (positive or negative), and creatinine, CA15-3 and alkaline phosphatase levels with overall survival. The variables with a statistically significant association with survival in the univariate analyses were selected for multivariate analyses (second phase).

In the univariate analyses, the best cutoff values for the continuous variables (whole skeletal SUVmean and SUVmax, age and creatinine, CA15-3 and alkaline phosphatase levels) to divide the patients in two groups with maximum survival difference between them were defined by maximally selected rank statistics (MSRS) using the Lausen test (*19*). The MSRS also provided the statistical level of significance (p-value) of the survival difference between the two groups. For the binary variables: presence of visceral metastases, histological subtypes, hormone receptor status and HER expression, the survival analyses between the two categories were performed using Kaplan-Meier curve and Log Rank test. Survival was measured from the date of the ^{18}F-NaF PET/CT and a significance level of $p \leq 0.05$ was adopted for statistical significance. However, variables with p value ≤ 0.1 in the univariate analyses could be selected for multivariate analysis.

Cox proportional hazard regression was used for multivariate survival analysis. In this analysis all variables were inserted as binary groups and the continuous variables were dichotomized using the cut-points defined in the Lausen test. A significance level of $p \leq 0.05$ was adopted for statistical significance.

For illustrative purposes, the Kaplan Meier curves of the variables that were statistically significant in the multivariate analysis were also presented.

It was also compared the time interval from diagnosis to the ^{18}F-NaF PET/CT studies between groups with low and high whole skeletal SUVmean and SUVmax, using unpaired T test.

The Lausen test was performed using R software version 3.6.1 and the other statistical analyses were done using SPSS® (version 20.0; IBM, Armonk, NY, USA). The descriptive results of the continuous variables were presented using the mean, the standard deviation (SD) and the range of the values.

Results

The characteristics of the patients and variables analyzed are presented in Table 1.
The mean follow up period was 966 days (SD: 606 days; Range 21 to 2921 days) and from the 176 patients analyzed 138 died during the follow up.

The results of the univariate analyses are presented in Table 2. In these analyses, the following variables presented a statistical significant association with overall survival ($p < 0.05$) and were selected for the multivariate analysis: CA15-3 and alkaline phosphatase levels, estrogen receptor status, whole skeletal mean and maximum SUV.

In the Cox regression analysis, high alkaline phosphatase level, the absence of estrogen receptors, and high whole skeletal mean and maximum SUVs were related to poor prognosis (Table 3). The Kaplan Meier curves of these variables are presented in Figure 4.

There were no statistically significant differences in the time from diagnosis to the ^{18}F-NaF PET/CT studies between groups with low and high whole skeletal SUVmean ($p = 0.82$) and SUVmax ($p = 0.79$).

Discussion

Skeletal metastatic disease is common in patients with breast cancer (*20*) and is a cause of morbidity and mortality in these patients (*21*). Moreover, not only the presence of bone metastases but also the number of metastatic sites has been associated with survival and some articles showed that patients with multiple skeletal metastatic sites present a shorter overall survival than patients with solitary bone metastasis (*22*,*23*). Radionuclide bone imaging has a main role in staging and follow up of patients with breast cancer due to its high sensitivity to detect skeletal metastasis, either with ^{99m}Tc-MDP (*24*) or ^{18}F-NaF PET/CT (*25*). However, the use of these methods to measure the burden of metastatic bone disease in patients with breast cancer is infrequent, possibly due to the scarcity of articles showing the association of this burden with prognosis and to the technical difficulties to use the methodologies of measurement in clinical practice.

For skeletal scintigraphy with ^{99m}Tc-MDP, there are studies showing the association of the bone scan index (BSI), a technique that calculates the amount (%) of bone metastases either manually (*26*) or, more recently, automatically (*27*), with the prognosis of the patients. But these articles are mainly on the use of the BSI in prostate cancer patients (*28*,*29*) and there are only a few articles about the use of BSI in breast cancer (*30*,*31*). A possible reason for this could be the different phenotypes of bone metastases in these cancers (sclerotic, lytic, or mixed), with a predominance of lytic pattern in breast cancer (*32*) and sclerotic in prostate cancer (*33*). Since

lytic lesions are less well visualized by bone scan, the BSI tends to underestimate the burden of disease in patients with breast cancer, which is not the case for prostate cancer where the lesions are almost all sclerotic, with a osteoblastic activity that is usually well characterized on radionuclide images.

For ^{18}F-NaF PET/CT studies, some articles have already described methodologies to measure the severity of bone metastatic disease and showed the association of this degree with prognosis. However, as for the BSI, most of the studies were done on patients with prostate cancer (*10,12,13*) and studies with other neoplastic diseases are less frequent (*14–17*). Particularly in breast cancer patients, few articles investigated the association of the burden of metastatic bone disease measured on ^{18}F-NaF PET/CT studies with prognosis. Brito et al. (*14*) analyzed a group of 49 patients with bone metastatic breast cancer and showed that the ones with higher burden of bone metastatic disease presented a worse prognosis. These authors further published an article validating a method for semiautomatic quantification of ^{18}F-NaF PET/CT studies (*11*). In this sense, our results also support the evidence that the burden of metastatic bone disease measured in ^{18}F-NaF PET/CT studies is associated with overall survival in patients with breast cancer. However, the methodology of quantification utilized in our study is different from that used in most of the studies published so far (*10–12*). While the last one is based on the definition of the metabolic boundaries of the metastatic lesions using SUV thresholds on the PET images, the methodology used in our study is based on a wsVOI defined in the CT image using HU thresholds and the further application of this wsVOI on the metabolic images. Nevertheless, this is not the first time this methodology is used and previous studies described its use to calculate the whole skeletal mean SUV in a group of patients with normal ^{18}F-NaF PET/CT studies (*34*) and to calculate the whole skeletal mean SUV in ^{18}F-NaF and ^{18}F-FDG PET/CT

studies in patients with multiple myeloma (*35*). However, this is the first time it was used in a group of patients with breast cancer.

Regarding the results of our study, it is worth highlighting that both SUV metrics (mean and maximum) were independently associated with overall survival. This result could be explained by the fact that while the whole skeletal maximum SUV represents the aggressiveness of the disease, the whole skeletal mean SUV represents the disease burden. This finding is partially in accordance with the scarce literature about this issue. The results of Brito et al. (*14*) showed that the burden of bone disease measured in the ^{18}F-NaF PET/CT studies is associated with overall survival in patients with bone metastatic breast cancer in the multivariate analysis while the SUVmax was only associated with survival in the univariate analysis. For patients with advanced genitourinary malignancies, Lim et al. (*17*) demonstrated that the burden of bone metastatic disease in the baseline ^{18}F-NaF PET/CT study is associated with OS while the SUVmax is associated with OS only in studies performed after therapies. For prostate cancer patients, Etchebehere et al. (*13*) showed that the burden of metastatic bone disease is associated with OS while the SUVmax is not. Therefore, future analysis should better evaluate the role of the SUV metrics in the prognostic assessment of patients with bone metastatic disease, particularly in patients with breast cancer.

Another interesting result of our study is that besides the whole skeletal SUV metrics, only the estrogen receptor status and the alkaline phosphatase level were associated with outcome in the multivariate analysis, while the CA15-3 level, that was associated with prognosis in the univariate analysis, did not present statistically significant association with survival in the multivariate analysis. The literature about the association of alkaline phosphatase and CA15-3 levels with breast cancer OS is controversial, with articles corroborating our findings and

showing the association of the alkaline phosphatase (*36*) but not of the CA 15-3 levels with prognosis (*37*), while others show the opposite finding, with absence of alkaline phosphatase association (*37*) and presence of CA 15-3 association with prognosis (*38*). The literature findings on hormonal receptors and HER-2 status are also heterogeneous, with some articles corroborating our findings that estrogen receptors are more important in the prognostic definition than the progesterone receptors (*39*) while others show that the relation among these parameters are complex and the prognosis will depend more on the distinct combination among them than on the isolate result of specific parameter (*40*). About the finding that the presence of soft tissue metastases was not associated with OS, even in the univariate analysis, this is in accordance with a previous article that showed that the burden of metastatic bone disease is more relevant than the presence of visceral metastases in prognosis in patients with breast cancer (*14*).

Thus, our results corroborate the importance of measuring the burden of metastatic bone disease as a prognostic indicator in patients with breast cancer since this burden can be one of the few variables independently associated with OS. Nevertheless, this study presents some limitations that should be reported. First, it is a retrospective study and we had not complete information on some variables that could have potentially confounded the results such as the burden and the sites of visceral metastatic sites, previous treatments and the stage of disease at the time of the diagnosis. Therefore, these variables were not part of the analysis and further studies will be necessary to know whether the addition of them would have changed the results. But, we did compare the time interval from diagnosis to ^{18}F-NaF PET/CT studies between groups with low and high whole skeletal SUVmean and SUVmax and it was not found differences in this parameter between groups. Consequently, this interval does not appear to be the cause of a higher burden of bone metastatic disease in our group of patients.

However, it is also important to explain that the main objective of our study was not to compare the burden of metastatic bone disease with all other potential prognostic variables in patients with breast cancer, especially because these variables are numerous and vary among different studies, but to assess the feasibility of a new methodology to estimate this burden and to analyze the performance of this methodology as a prognostic indicator in a group of patients with breast cancer. Second, we did not perform a comparison between the methodology used in our study and the ones utilized in previous studies. The methodology used in our study can have potential advantages such as not being dependent on SUV levels to define the lesions, which could be particularly favorable in breast cancer where the presence of lytic lesions with low uptake are common with limitations in their detection using SUV thresholds. On the other hand, the whole skeletal mean SUV is a composition of the uptake degree in the malignant lesions as well as in benign lesions and normal bone, which is particularly disadvantageous in patients with extensive osteo degenerative bone disease and low burden of metastatic disease. Nevertheless, the method based on the whole skeletal mean SUV could be further improved by erasing the areas with benign uptake from the metabolic images before the wsVOI is applied to it. Thus, future analyses on the main advantages, disadvantages and potential complementarity of these methodologies should be performed.

Last, we should remember that the ^{18}F-NaF and the ^{99m}Tc-MDP uptake occurs mainly in cortical bone metastases, while the metastatic lesions initially appear in the bone marrow before invade the cortical. Therefore, if we wish to proper quantify the burden of metastatic skeletal disease using tracers that also present uptake in bone marrow lesions, such as ^{18}F-FDG and ^{18}F-fluoroestradiol, other segmentation techniques not based strictly on HU might be better suited.

Conclusion

The results showed that in our group of patients with bone metastatic breast cancer, the calculation of the whole skeletal mean SUV in ^{18}F-NaF PET/CT studies is feasible and it is an independent predictor of overall survival.

Disclosure

The authors warrant that they do not have conflicts of interest and that they have not received any financial support for this study such as grants, consulting fees, travel fees, or honoraria.

Key points

Question:

Is the whole skeletal mean standardized uptake value in 18F-NaF PET/CT studies associated with overall survival in patients with bone metastatic breast cancer?

Pertinent Findings:

It is feasible to create whole skeletal volumes of interest (wsVOIs) based on the CT component of the PET/CT study using Hounsfield unit thresholds. These wsVOIs can then be applied to the metabolic component of the 18F-NaF PET/CT studies to calculate the whole skeletal mean standardized uptake value of the patients.

In a retrospective cohort analysis, it was found that in a group of patients with bone metastatic breast cancer the whole skeletal mean standardized uptake value is an independent predictor of overall survival.

Implications for Patient Care:

The findings of this study can be useful to provide better prognostic information to patients with bone metastatic breast cancer.

References

1. Tateishi U, Morita S, Taguri M, et al. A meta-analysis of (18)F-Fluoride positron emission tomography for assessment of metastatic bone tumor. *Ann Nucl Med*. 2010;24:523-531.

2. Iagaru A, Young P, Mittra E, Dick DW, Herfkens R, Gambhir SS. Pilot prospective evaluation of 99mTc-MDP scintigraphy, 18F NaF PET/CT, 18F FDG PET/CT and whole-body MRI for detection of skeletal metastases. *Clin Nucl Med*. 2013;38:e290-6.

3. Even-Sapir E, Metser U, Mishani E, Lievshitz G, Lerman H, Leibovitch I. The detection of bone metastases in patients with high-risk prostate cancer: 99mTc-MDP Planar bone scintigraphy, single- and multi-field-of-view SPECT, 18F-fluoride PET, and 18F-fluoride PET/CT. *J Nucl Med*. 2006;47:287-297.

4. Jambor I, Kuisma A, Ramadan S, et al. Prospective evaluation of planar bone scintigraphy, SPECT, SPECT/CT, 18F-NaF PET/CT and whole body 1.5T MRI, including DWI, for the detection of bone metastases in high risk breast and prostate cancer patients: SKELETA clinical trial. *Acta Oncol*. 2016;55:59-67.

5. Löfgren J, Mortensen J, Rasmussen SH, et al. A prospective study comparing Tc-hydroxyethylene-diphosphonate planar bone scintigraphy and whole-body SPECT/CT with 18F-fluoride PET/CT and 18F-fluoride PET/MRI for diagnosing bone metastases. *J Nucl Med*. 2017;58:1778-1785.

6. Hellwig D, Marienhagen J, Menhart K, Grosse J. [Nuclear Medicine in Germany. Updated key data and trends from official statistics]. *Nuklearmedizin*. 2017;56:55-68.

7. Páez D, Orellana P, Gutiérrez C, Ramirez R, Mut F, Torres L. Current status of nuclear medicine practice in latin america and the caribbean. *J Nucl Med*. 2015;56:1629-1634.

8. Segall GM. PET/CT with sodium 18F-fluoride for management of patients with prostate cancer. *J Nucl Med*. 2014;55:531-533.

9. Hillner BE, Siegel BA, Hanna L, et al. Impact of (18)F-fluoride PET on intended management of patients with cancers other than prostate cancer: Results from the national oncologic PET registry. *J Nucl Med*. 2014;55:1054-1061.

10. Rohren EM, Etchebehere EC, Araujo JC, et al. Determination of skeletal tumor burden on 18F-fluoride PET/CT. *J Nucl Med*. 2015;56:1507-1512.

11. Brito AE, Mourato F, Santos A, Mosci C, Ramos C, Etchebehere E. Validation of the semiautomatic quantification of F-fluoride PET/CT whole-body skeletal tumor burden. *J Nucl Med Technol*. 2018;46:378-383.

12. Lapa P, Marques M, Costa G, Iagaru A, Pedroso de Lima J. Assessment of skeletal tumour burden on 18F-NaF PET/CT using a new quantitative method. *Nucl Med Commun*. 2017;38:325-332.

13. Etchebehere EC, Araujo JC, Fox PS, Swanston NM, Macapinlac HA, Rohren EM. Prognostic factors in patients treated with 223Ra: The role of skeletal tumor burden on baseline 18F-fluoride PET/CT in predicting overall survival. *J Nucl Med*. 2015;56:1177-1184.

14. Brito AE, Santos A, Sasse AD, et al. 18F-Fluoride PET/CT tumor burden quantification predicts survival in breast cancer. *Oncotarget*. 2017;8:36001-36011.

15. Ueda CE, Duarte PS, de Castroneves LA, et al. Burden of metastatic bone disease measured on 18F-NaF PET/computed tomography studies as a prognostic indicator in patients with medullary thyroid cancer. *Nucl Med Commun*. 2020;41:469-476.

16. Zadeh MZ, Seraj SM, Østergaard B, et al. Prognostic significance of 18F-sodium fluoride in newly diagnosed multiple myeloma patients. *Am J Nucl Med Mol Imaging*. 2020;10:151-160.

17. Lim I, Lindenberg ML, Mena E, et al. 18F-Sodium fluoride PET/CT predicts overall survival in patients with advanced genitourinary malignancies treated with cabozantinib and nivolumab with or without ipilimumab. *Eur J Nucl Med Mol Imaging*. 2020;47:178-184.

18. Loening AM, Gambhir SS. AMIDE: a free software tool for multimodality medical image analysis. *Mol Imaging*. 2003;2:131-137.

19. Lausen B, Schumacher M. Maximally Selected Rank Statistics. *Biometrics*. 1992;48:73.

20. Lee YT. Breast carcinoma: Pattern of metastasis at autopsy. *J Surg Oncol*. 1983;23:175-180.

21. Coleman RE, Rubens RD. The clinical course of bone metastases from breast cancer. *Br J Cancer*. 1987;55:61-66.

22. Koizumi M, Yoshimoto M, Kasumi F, Ogata E. Comparison between solitary and multiple skeletal metastatic lesions of breast cancer patients. *Ann Oncol*. 2003;14:1234-1240.

23. Parkes A, Warneke CL, Clifton K, et al. Prognostic factors in patients with metastatic breast cancer with bone-only metastases. *Oncologist*. 2018;23:1282-1288.

24. Costelloe CM, Rohren EM, Madewell JE, et al. Imaging bone metastases in breast cancer: techniques and recommendations for diagnosis. *Lancet Oncol*. 2009;10:606-614.

25. Yoon S-H, Kim KS, Kang SY, et al. Usefulness of (18)F-fluoride PET/CT in breast cancer patients with osteosclerotic bone metastases. *Nucl Med Mol Imaging* . 2013;47:27-35.

26. Erdi YE, Humm JL, Imbriaco M, Yeung H, Larson SM. Quantitative bone metastases analysis based on image segmentation. *J Nucl Med*. 1997;38:1401-1406.

27. Nakajima K, Nakajima Y, Horikoshi H, et al. Enhanced diagnostic accuracy for quantitative bone scan using an artificial neural network system: a Japanese multi-center database

project. *EJNMMI Res*. 2013;3:83.

28. Ulmert D, Kaboteh R, Fox JJ, et al. A novel automated platform for quantifying the extent of skeletal tumour involvement in prostate cancer patients using the Bone Scan Index. *Eur Urol*. 2012;62:78-84.

29. Nakajima K, Edenbrandt L, Mizokami A. Bone scan index: A new biomarker of bone metastasis in patients with prostate cancer. *Int J Urol*. 2017;24:668-673.

30. Idota A, Sawaki M, Yoshimura A, et al. Bone Scan Index predicts skeletal-related events in patients with metastatic breast cancer. *Springerplus*. 2016;5:1095.

31. Iwase T, Yamamoto N, Ichihara H, Togawa T, Nagashima T, Miyazaki M. The relationship between skeletal-related events and bone scan index for the treatment of bone metastasis with breast cancer patients. *Medicine*. 2014;93:e269.

32. Glendenning J, Cook G. Imaging breast cancer bone metastases: current status and future directions. *Semin Nucl Med*. 2013;43:317-323.

33. Cook GJR, Azad G, Padhani AR. Bone imaging in prostate cancer: the evolving roles of nuclear medicine and radiology. *Clin Transl Imaging*. 2016;4:439-447.

34. Gomes Marin JF, Duarte PS, Willegaignon de Amorim de Carvalho J, Sado HN, Sapienza MT, Buchpiguel CA. Comparison of the variability of SUV normalized by skeletal volume with the variability of SUV normalized by body weight in 18F-Fluoride PET/CT. *J Nucl Med Technol*. 2019;47:60-63.

35. Zadeh MZ, Raynor WY, Seraj SM, et al. Evolving roles of fluorodeoxyglucose and sodium fluoride in assessment of multiple myeloma patients: Introducing a novel method of PET quantification to overcome shortcomings of the existing approaches. *PET Clin*. 2019;14:341-352.

36. Chen B, Dai D, Tang H, et al. Pre-treatment serum alkaline phosphatase and lactate dehydrogenase as prognostic factors in triple negative breast cancer. *J Cancer*. 2016;7:2309-2316.

37. Nieder C, Dalhaug A, Haukland E, Mannsaker B, Pawinski A. Prognostic impact of the tumor marker CA 15-3 in patients with breast cancer and bone metastases treated with palliative radiotherapy. *J Clin Med Res*. 2017;9:183-187.

38. Li X, Dai D, Chen B, Tang H, Xie X, Wei W. Clinicopathological and prognostic significance of cancer antigen 15-3 and carcinoembryonic antigen in breast cancer: A meta-analysis including 12,993 patients. *Dis Markers*. 2018;2018:9863092.

39. Lower EE, Glass EL, Bradley DA, Blau R, Heffelfinger S. Impact of metastatic estrogen receptor and progesterone receptor status on survival. *Breast Cancer Res Treat*. 2005;90:65-70.

40. Lv M, Mao Y, Song Y, et al. Clinical features and survival of single hormone receptor-positive breast cancer: A population-based study of 531,605 patients. *Clin Breast Cancer*. 2020;20:e589-e599.

Legends

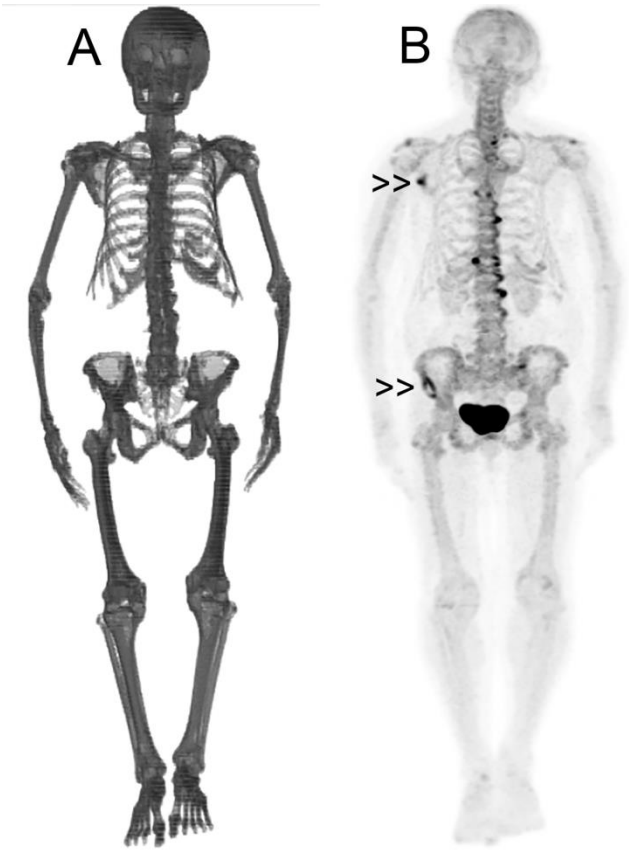

Figure 1: wsVOI (A) and ^{18}F-NaF PET/CT coronal maximum intensity projection image (B) of a patient with few metastatic sites in the skeletal (double arrow head). The patient presented a whole skeletal mean SUV of 2.20 and still alive 1974 days after the study.

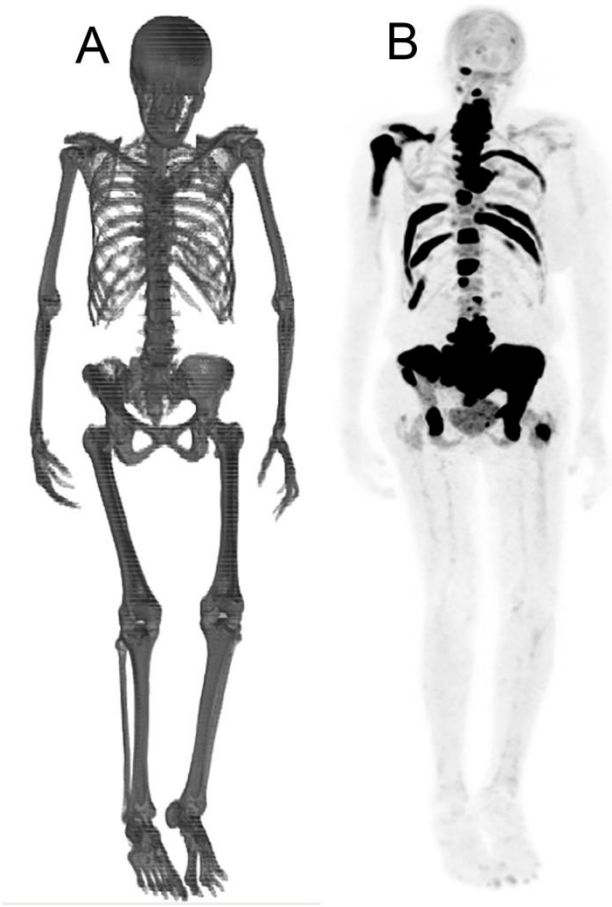

Figure 2: wsVOI (A) and ^{18}F-NaF PET/CT coronal maximum intensity projection image (B) of a patient with multiple metastatic sites in the skeletal. The patient presented a whole skeletal mean SUV of 3.58 and died 153 days after the study.

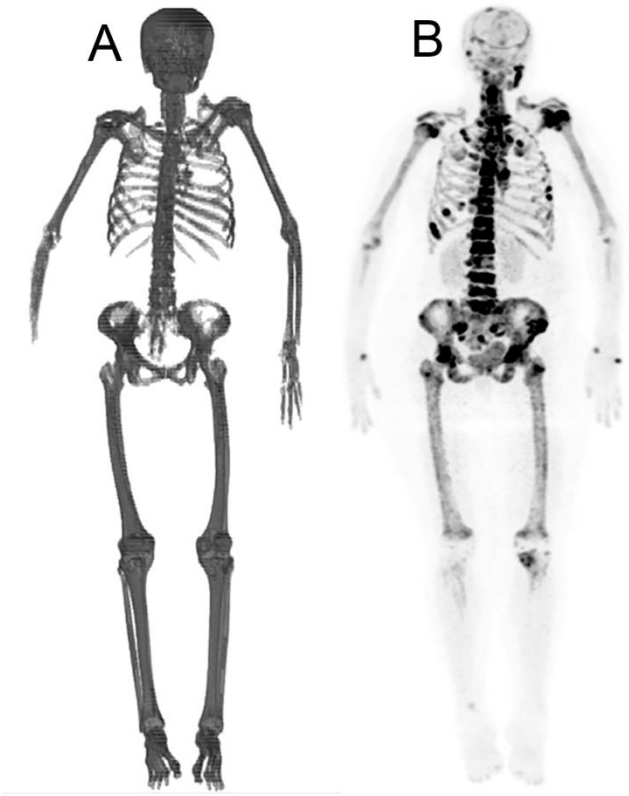

Figure 3: wsVOI (A) and ^{18}F-NaF PET/CT coronal maximum intensity projection image (B) of a patient with widespread bone metastatic disease characterized by a diffuse and heterogeneous uptake of the radiopharmaceutical in the axial and proximal appendicular skeleton. The patient presented a whole skeletal mean SUV of 4.78 and died 66 days after the study. In this case the wsVOI was not capable of detecting the right hand and some fingers of the left hand.

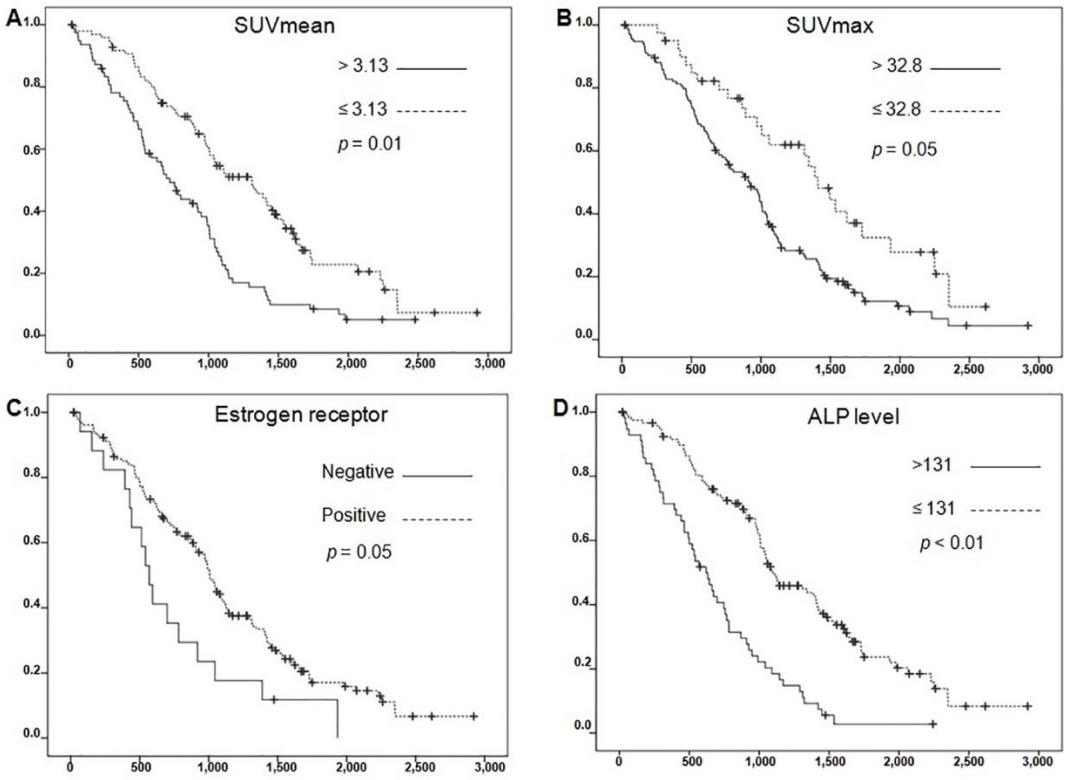

Figure 4: Kaplan Meier curves for the variables whole skeletal SUVmean (A) and SUVmax (B), estrogen receptor status (C) and alkaline phosphatase level (D). The y axis displays the survival probability and the x axis the survival time in days. The cutoff values for the division of the variables in two comparison groups are presented in Table 2 and in the figure. The statistical significance of the survival differences between the groups are presented in Table 3 and in the figure. + = censored patients. ALP = alkaline phosphatase level

Table 1: Characteristics of the patients and of the variables analyzed.

Variables	**Mean**	**SD**	**Min**	**Max**
Follow-up time (days)	941.9	578.4	21	2711
wsSUVmax	48.5	22.6	6.2	144.2
wsSUVmean	3.2	0.9	0.3	6.1
Age (years)	56.9	13.4	27.4	88.2
Creatinine level (mg/dL)	0.7	0.3	0.3	2.7
CA15-3 level (units/mL)	438.1	1412.4	7.0	12000.0
ALP level (units/L)	142.1	133.8	21.1	955.0
Diagnosis to PET (days)	1194.7	1451.9	-1*	6794
	Absent (%)	**Present (%)**		**Missing (%)**
VM	131 (74)	45 (26)		0 (0)
Estrogen receptor	18 (10)	156 (89)		2 (1)
Progesterone receptor	41 (23)	130 (74)		5 (3)
HER-2	139 (79)	35 (20)		2 (1)
	IDC (%)	**ILC (%)**	**Other (%)**	**Missing (%)**
Histological Subtype	153 (87)	14(8)	4(2.3)	5(2.8)

*wsSUVmax = whole skeletal maximum SUV; wsSUVmean = whole skeletal mean SUV; SD = standard deviation; Min = minimum; Max = maximum; VM = visceral metastases; ALP = alkaline phosphatase; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; HER-2 = human epidermal growth factor receptor 2; * The ^{18}F-NaF PET/CT was performed before the result of the pathological study confirmed the cancer diagnosis.*

Table 2: Results of the univariate analyses. The variables were divided in two groups for survival comparison. The numbers of individuals in group 1, and the p values of the difference in survival between the two groups are also presented.

Variables	**Group 1**	**Group 2**	**N in group 1 (%)**	**p-Value**
wsSUVmax	≤ 32.8	> 32.8	42 (24)	0.02*
wsSUVmean	≤ 3.13	> 3.13	97 (55)	< 0.01*
Age (years)	≤ 56	> 56	91 (52)	0.48*
Creatinine (mg/dL)	≤ 0.83	> 0.83	135 (77)	0.57*
CA15-3 (units/mL)	≤ 19.0	> 19.0	20 (11)	0.02*
ALP (units/L)	≤ 131	> 131	118 (67)	< 0.01*
VM	Absent	Present	131 (74)	0.15†
Estrogen receptor	Absent	Present	18 (10)‡	0.01#
Progesterone receptor	Absent	Present	41 (24)‡	0.77†
HER-2	Absent	Present	139 (80)‡	0.24†
Histological Subtype	IDC	ILC	153 (92%)‡	0.40†

*wsSUVmax = whole skeletal maximum SUV; wsSUVmean = whole skeletal mean SUV; VM = visceral metastases; ALP = alkaline phosphatase; IDC = invasive ductal carcinoma; ILC = invasive lobular carcinoma; HER-2 = human epidermal growth factor receptor 2; * = p value calculated using Lausen test; † = p value calculated using Log Rank test; ‡ = percentage in relation to the two groups compared (excluding missing data and other groups). Highlighted in grey are the variables selected for the multivariate analyses (p ≤ 0.1).*

Table 3: Results of the cox regression evaluating the association of the variables selected in the univariate analyses with survival.

Variable	Group	HR	95% CI	p-Value
wsSUVmax	> 32.8	1.60	1.00 - 2.57	0.05
wsSUVmean	> 3.13	1.57	1.10 - 2.24	0.01
CA15-3 level (units/mL)	> 19.0	1.26	0.66 - 2.42	0.48
ALP level (units/L)	> 131	2.14	1.47 - 3.12	<0.01
Estrogen receptor	Present	0.59	0.35 - 1.01	0.05

wsSUVmax = whole skeletal maximum SUV; wsSUVmean = whole skeletal mean SUV; ALP = alkaline phosphatase; HR = hazard ratio; CI = confidence interval. Highlighted in grey are the independent variables on Cox regression ($p \leq 0.05$).

Graphical Abstract