Diagnosis, Treatment Response and Prognosis. The role of \textsuperscript{18}F-DOPA PET/CT in children affected by Neuroblastoma in comparison with \textsuperscript{123}I-mIBG scan. The first prospective study.

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ABSTRACT

Purpose

To evaluate the diagnostic role of 18F-DOPA PET/CT at the time of staging, in children with Neuroblastoma (NB) and to investigate its ability to assess treatment response. We also investigated the prognostic value of 18F-DOPA PET/CT at the same time-points.

Methods

We enrolled children with NB at onset. Before and after induction chemotherapy, all patients underwent 18F-DOPA PET/CT and 123I-mIBG scan plus SPECT/CT. 18F-DOPA PET/CT results were compared with those of 123I-mIBG WBS. For each modality, patient-based (PBA) and lesion-based analyses (LBA) were performed and sensitivity was calculated. We applied scoring systems to 123I-mIBG scan and 18F-DOPA PET/CT (i.e. 123I-mIBG whole body score (WBS) and whole-body metabolic burden (WBMB), respectively) and evaluated the association between these parameters, the principal NB risk-factors and outcome.

Results

We enrolled 16 high- and 2 intermediate-risk NB patients. On PBA, the sensitivity of 123I-mIBG WBS and 18F-DOPA PET/CT in detecting primary tumours, soft tissue and bone/bone-marrow metastases was 83%, 50% and 92%, versus 94%, 92% and 100%, respectively. On LBA, the sensitivity of 18F-DOPA PET/CT in detecting soft tissue and bone/bone-marrow metastases was 86% and 99% - significantly higher than that of 123I-mIBG WBS: 41% and 93%.

After therapy, on PBA, the sensitivity of 123I-mIBG WBS and 18F-DOPA PET/CT in detecting primary tumours, soft tissue and bone/bone-marrow metastases was 72%, 33% and 38%, versus 83%, 75% and 54%, respectively. On LBA, the sensitivity of 18F-DOPA PET/CT in detecting soft tissue and bone/bone-marrow metastases was 77% and 86% - significantly higher than that of 123I-mIBG WBS (28% and 69%).

During follow-up, 8 cases of disease progression and 5 deaths occurred. On multivariate analysis, only post-therapeutic 18F-DOPA WBMB (>7.5) was associated to progression-free survival.

Conclusion

18F-DOPA PET/CT is more sensitive than 123I-mIBG WBS in staging NB patients and evaluating disease persistence after chemotherapy. In a time-to-event analysis, post-therapeutic 18F-DOPA WBMB remained the only risk factor associated to disease progression.
INTRODUCTION

High-risk neuroblastoma (HR-NB), commonly defined by metastatic disease at above 12–18 months of age (1,2), by proto-oncogene MYCN (MYCNA) amplification at any age, and by unfavourable histopathologic features (3, 4), displays long-term survival rates of approximately 40% (5–7). Internationally agreed treatment options for HR-NB (NB-AR-01 protocol) include multi-agent induction chemotherapy, surgery, high-dose chemotherapy followed by autologous stem cell transplantation, external beam radiotherapy, radionuclide therapy, differentiation therapies, and immunotherapy (8). Complete response to induction chemotherapy, as evaluated by various diagnostic procedures (e.g. imaging or bone-marrow biopsy), is one of the main early prognostic factors in HR-NBL.

123I-meta-iodobenzylguanidine (123I-mIBG) scintigraphy has long been recognized as the main imaging procedure for staging NBL. More recently, the International Society of Paediatric Oncology European Neuroblastoma Group (SIOPEN) identified the SIOPEN semi-quantitative mIBG skeletal scoring system as an excellent inter- and intra-observer method that can estimate the extension of bone-bone marrow metastases in a reproducible way, thereby providing a reliable prognostic indicator in NB (9). In addition, the SIOPEN scoring system was validated as a prognostic response predictor in two independent trial populations (8). More specifically, a post-induction skeletal score > 3 identifies patients with very poor prognosis who require different treatment strategies (9). On the other hand, 123I-mIBG scintigraphy is cumbersome (10), requiring proper patient preparation and correct recognition of the distribution pattern, and is frequently conditioned by the difficult anatomy in children and low-quality images (11). Various PET tracers have been tested as effective substitutes for 123I-mIBG in assessing NB (12), and 18F-DOPA (13), radiolabelled somatostatin analogues (14,15) and 124I-mIBG have proved the most promising (16). 18F-DOPA PET/CT has already proved to be more sensitive than 123I-mIBG scan in patients with NB relapse (17). Specifically, 18F-DOPA PET/CT has been demonstrated to be a reliable diagnostic procedure in detecting small soft tissue and bone/bone marrow metastases that are not accurately detected by 123I-mIBG scintigraphy (17,18). In addition, this imaging procedure has shown better diagnostic results than conventional procedures (i.e. CT and MRI), disclosing more NB localizations in bone marrow, lymph nodes and soft tissue recurrences (19). Direct comparison with 18F-FDG PET/CT has also shown that 18F-DOPA PET/CT is the most sensitive and accurate method of identifying NB localizations, whether metastases or primary tumours (20). Moreover, its prognostic role at the time of recurrence has
already been validated by means of an adequate whole-body scoring system, defined as whole-body metabolic burden (WBMB), which is able to evaluate disease extension (18). In this context, the possibility of disease progression and death has been seen to increase in proportion to the WBMB value (with a cut-off value of >7.5) (18). However, 18F-DOPA PET/CT has never been tested as a pivotal diagnostic tool for staging patients affected by NB and evaluating treatment response to induction chemotherapy. Indeed, this diagnostic PET-based approach could help in stratifying patients after induction chemotherapy and identify subjects at different risk of disease persistence/recurrence. The proven ability of 18F-DOPA PET/CT to detect small metastases could be even more important when evaluating disease persistence.

The primary aim of this study was to evaluate the diagnostic role of 18F-DOPA PET/CT at the time of first diagnosis in children affected by NB. We also investigated the ability of this procedure to assess response to chemotherapy. Lastly, we evaluated the prognostic role of 18F-DOPA PET/CT in high-risk NB patients on diagnosis and after induction chemotherapy by testing the relationship between WBMB, progression-free survival (PFS) and overall survival (OS).
MATERIALS and METHODS

The local ethics committee and the “Agenzia Italiana del Farmaco”, a public agency of the Italian Ministry of Health, have approved this study. Written informed consent was obtained from all subjects. The trial was registered in the European Clinical Trial Database (EudraCT number 2012-005398-30).

Patient Population

From December 2013 to January 2017, we prospectively enrolled NB patients who were candidates for SIOPEN therapeutic protocols and referred for imaging procedures at the Nuclear Medicine Department of E.O. Galliera Hospital (Genoa-Italy). Before and within 7 days after chemotherapy all patients underwent both 18F-DOPA PET/CT and 123I-mIBG whole-body scan (WBS) with additional SPECT. The imaging procedures were performed within 10 days of each other (range 1-10, mean 4.1, SD +/- 3.3).

Inclusion criteria were:

- histologically proven Neuroblastoma at the time of first diagnosis (age > 12 months and < 18 years).
- no previous chemotherapy treatment.
- written informed consent obtained.

Exclusion criteria were:

- comorbidity due to other neoplasms
- known hypersensitivity to the active ingredient or excipients contained in the radiopharmaceuticals
- any other medical condition contraindicating the study in the investigator's judgment.

Imaging modalities

123I-mIBG scintigraphy and 18F-DOPA PET/CT were performed on fasting patients within 10 days of each other; no treatment was administered between the two scans. Images were acquired according to standard procedures (10).
Whole-body $^{18}$F-DOPA PET/CT was carried out 60 min after tracer injection according to our previous experience (10, 17-19). The activity administered was calculated according to the patient’s body weight (4 MBq/kg) with a minimum activity of 80 MBq (range 80-185, mean 110, SD +/- 35).

$^{123}$I-mIBG scans were acquired 24 h after injection of the tracer by means of a dual-head gamma camera (Millennium, GE Medical Systems, Milwaukee, WI, USA). The activity administered was calculated according to the patient’s body weight, with a minimum activity of 80 MBq, as suggested by Lassmann et al. (range 80-185, mean 110, SD +/- 35) (21). Spot views of the various body segments were acquired. Each spot view was acquired for a maximum of 10 min (about 500 kcounts; 100 kcounts for spot views of the lower limbs). Thoraco-abdominal single photon emission computed tomography (SPECT) was performed in all patients at intervals of 24 h, as suggested by Matthay et al. and Olivier et al (22,23). Fused SPECT/CT images were analysed on a dedicated workstation (Xeleris GE) which allowed co-registration of mIBG and CT images previously acquired during PET/CT examination.

**Image Interpretation**

Two expert nuclear medicine physicians, working separately, interpreted $^{18}$F-DOPA PET/CT and $^{123}$I-mIBG SPECT/CT. They were aware of the patient’s clinical history and anatomical imaging modalities (i.e. MRI/CT) but each was blinded to the interpretation of the other. Finally, any discordance was resolved by consensus.

On $^{18}$F-DOPA PET/CT or $^{123}$I-mIBG SPECT/CT any focal, non-physiological uptake higher than that of the surrounding background was considered pathological (17,18). The two readers revised the whole-body images by focusing on primary tumours (abdominal or thoracic), lymph nodes, lungs, liver, bone and brain. Both studies were interpreted on a patient-by-patient (PBA) and lesion-by-lesion basis (LBA) before and after chemotherapy. In PBA, detection rate (DR) was defined as the ability to detect at least one pathological finding in each subject. In LBA, the DR was defined as the ability to detect suspect lesions in relation to the total number of lesions detected by both tracers and by anatomical imaging modalities.

**Scoring systems**

The effectiveness of $^{123}$I-mIBG and $^{18}$F-DOPA PET/CT in detecting NB was assessed by reviewing the uptake patterns for each radiopharmaceutical in the following locations: primary tumour, local and regional soft tissue
metastases, and bone and bone marrow metastases. A semiquantitative scoring-system for NB, the SIOPEN method
3 scoring system, was applied to the 123I-mIBG scan in order to evaluate disease extent in the bone and bone marrow
(8). To semi-quantify soft tissue NB localizations, the modified Curie scoring system was applied, based upon the
methodology of Matthay et al. (soft tissue123I-mIBG score) (18,24).

Another scoring system, the WBMB, was applied to 18F-DOPA PET/CT to evaluate the extension of bone/bone
marrow involvement and that of primary tumour and of soft-tissue metastases (18). Specifically: for 18F-DOPA
PET/CT, the SIOPEN method 3 scoring system was applied to evaluate the extent of bone and bone marrow disease.
To better characterize the intrinsic metabolic burden of each bone segment (B-MB), we multiplied the mean
standardized uptake value (SUVmean) by the score of each bone segment. The whole-body bone metabolic burden
(WB-B-MB) was calculated as the sum of the B-MB of each bone segment in the PET image. To determine the extent
and the load of soft tissue recurrence/metastases, a whole-body soft tissue metabolic burden (WB-S-MB) was applied
per patient (18,25). For each tumour lesion, the soft tissue metabolic burden (S-MB) was calculated as: S-MB=
SUVmean X tumour volume. Tumour volume was obtained from the CT images of the PET/CT acquisitions (26).
The WB-S-MB was calculated as the sum of the MB of each tumour lesion in the PET image. Finally, the overall
whole-body metabolic burden (WBMB) was calculated as the sum of WB-B-MB+WB-S-MB.

Risk stratification

Each patient was risk stratified according to: age at the time of diagnosis (≤18 or >18 months), histopathology, stage
(stage 3 or 4), MYCN amplification, lactate dehydrogenase (LDH), Homovanillic and Vanilmandelic acid levels, mIBG
score measured before and after induction chemotherapy and WBMB measured before and after induction
chemotherapy.

At final follow-up, patients were deemed to be disease free if they had less than 10 mm residual soft tissue at primary
site and non-primary lesions and complete resolution of mIBG uptake (27).

Patients were considered to have persistence of disease/partial response if there was a decrease of more than 30% of
the primary and non-primary site and mIBG uptake at primary site stable, improved, or resolved and more than 50%
reduction in mIBG absolute bone score (27).
Progression of disease was considered if there was a 20% increase in longest diameter or any new soft tissue or bone lesions detected by CT/MRI that was also mIBG avid or that was biopsied and confirmed to be Neuroblastoma (27).

**Standard of reference**

Although only DRs were calculated for each diagnostic modality, we applied a standard of reference, which was able to provide some confirmation of the site of disease. The standard of reference for primary tumour was based on histopathology (available in all patients). The standard of reference for soft tissue metastases was based on histopathology and/or diagnostic contrast-enhanced CT and/or MRI findings (available in all patients). The gold standards for bone and bone marrow metastases were bone marrow biopsy (available in all patients), CT and/or MRI (available in all patients). A median clinical and imaging follow-up time of 29.3 months, (range 19–53) was available for each patient.

The clinical decisions making after induction chemotherapy was based on mIBG WBS and conventional radiological imaging (i.e. CT and MRI) and did not consider 18F-DOPA PET/CT results.

**Statistical analysis**

Descriptive statistics included mean, standard deviation (SD) and range in case of continuous factors and scores; in the case of categorical factors, we used absolute and relative frequencies (%). Spearman’s rank correlation coefficient was adopted to test the correlation between $^{123}$I-mIBG SPECT/CT and $^{18}$F-DOPA PET/CT scores. For diagnostic analyses, detection rates (DR), defined as percentage of the number of positive subjects (in patient-based analysis) or lesion (in lesion-based analysis) on the total number of patients (or lesions) were calculated for each single diagnostic modality in each site of disease. We adopted the exact McNemar test to compare the DRs between diagnostic modalities. For prognostic analyses, we used Kaplan-Meier estimates of the cumulative probability of PFS and OS, defined as the interval between initial diagnosis and the onset of disease persistence/progression or death, and we tested the difference between time-to-event curves by means of the log-rank test. Cox proportional hazard modelling was used to estimate the risk of disease persistence/progression and death from any cause adjusting for age and other factors which proved to be associated to PFS and OS at univariate analyses. Since $^{123}$I-mIBG SPECT/CT and $^{18}$F-DOPA PET/CT scores were highly correlated, to avoid collinearity we used different models for each score to test
their independent association with PFS and OS. All analyses were conducted by means of Stata (version 14.2, StataCorp., College Station, TX, USA) software. Two-tailed probabilities are reported and a p-value of 0.05 was used to define nominal statistical significance.
RESULTS

We enrolled 16 HR-NB and 2 intermediate-risk patients; their main characteristics are summarized in Table 1. For clinical reasons, the 2 intermediate-risk patients and 1 HR-NB did not undergo NB-AR-01, but different therapeutic protocols with different schemes of induction therapy; they were therefore included only in the diagnostic analysis and excluded from the prognostic analysis (Table 1).

At the time of first staging, $^{123}$I-mIBG SPECT/CT proved positive in 17 out of 18 NB patients, while $^{18}$F-DOPA PET/CT was positive in all 18 patients. On PBA, the DR of $^{123}$I-mIBG SPECT/CT and $^{18}$F-DOPA PET/CT in detecting primary tumours, soft tissue metastases and bone/bone marrow metastases was 83%, 50%, and 92% versus 94%, 92% and 100%, respectively (Fig. 1 and Table 2). On LBA, the DR of $^{18}$F-DOPA PET/CT in detecting soft tissue and bone/bone marrow metastases was 86% and 99%: significantly higher (p<0.001) than that of $^{123}$I-mIBG SPECT/CT (41% and 93%) (Fig. 2 and Table 3).

After induction chemotherapy, $^{123}$I-mIBG SPECT/CT proved positive in 14 out of 18 NB patients, while $^{18}$F-DOPA PET/CT was still positive in 17 patients. However, when the analysis did not consider primary tumours but only metastatic sites of disease, $^{123}$I-mIBG SPECT/CT proved positive in only 8 patients; by contrast, $^{18}$F-DOPA PET/CT was still positive in 15.

On PBA, the DR of $^{123}$I-mIBG SPECT/CT and $^{18}$F-DOPA PET/CT in detecting primary tumours, soft tissue metastases and bone/bone marrow metastases was 72%, 33%, and 38% versus 83%, 75% and 54%, respectively (Table 2). On LBA, the DR of $^{18}$F-DOPA PET/CT in detecting soft tissue and bone/bone marrow metastases was 77% and 86%: significantly higher than that of $^{123}$I-mIBG SPECT/CT (28% and 69%, p<0.001 and p=0.001, respectively) (Table 3 and Fig. 3).

Over a median follow-up of 29.3 months (interquartile range: 19.0–53.1), among the 15 patients considered in the prognostic analysis, 8 cases of disease progression and 5 deaths occurred. Kaplan-Meier PFS curves showed that only initial stage (Stage 3 vs 4) and the $^{18}$F-DOPA WBMB measured after induction chemotherapy (WBMB>7.5) (Fig. 4) were associated with prognosis. However, an important trend towards significance was also observed between outcome and pre-therapy mIBG-WBS ($\geq 46$), pre-therapy $^{18}$F-DOPA WBMB ($\geq 45$) (Fig. 4) and homovanillic acid.
levels (≥32 umol/mmol creatinine) measured before therapy. Finally, risk estimates (unadjusted and adjusted) for
disease progression were also calculated from the Cox model. After adjustment for all risk factors showing at least a
trend towards significance at the univariate level, \(^{18}\text{F-DOPA WBMB}\) evaluated after induction chemotherapy was the
only factor independently and directly associated to PFS. More specifically, patients with \(^{18}\text{F-DOPA WBMB}\) score
>7.5 displayed a significantly higher risk of disease progression than those with \(^{18}\text{F-DOPA WBMB} \leq 7.5\) [hazard ratio
(HR) 10.7, 95% confidence interval (CI) 1.09–104.8, \(p=0.041\)]. No significant association was found between the risk
factors considered and OS.

Of the nine patients with completely negative mIBG-WBS and persistently positive \(^{18}\text{F-DOPA WBMB}\) after induction
chemotherapy, 3 showed persistence or progression of disease at the end of treatment. However, the 6 patients who
did not develop disease progression had an \(^{18}\text{F-DOPA WBMB}\) value no higher than 1. All 5 patients with WBMB
>7.5 after induction chemotherapy developed persistence/progression of disease, while only 2 of the 10 patients with
WBMB < 7.5 developed persistence/progression of disease (Supplemental Fig. 1). By contrast, although a value of
mIBG-WBS >3 identified 3 patients who showed disease persistence/progression, 4 (33%) of the 12 patients with
mIBG-WBS \(\leq 3\) developed disease persistence/progression (Fig. 5). Thus, \(^{123}\text{I-mIBG WBS}\) and \(^{18}\text{F-DOPA WBMB}\) scores were positively correlated only before induction chemotherapy (pre: Spearman rho=0.61, \(p=0.007\); post:
rho=0.38, \(p=0.11\)). Specifically, \(^{18}\text{F-DOPA WBMB}\) showed greater dispersion than \(^{123}\text{I-mIBG WBS}\), displaying an
interquartile range from 20 to 128 before chemotherapy and from 1 to 9 and after chemotherapy, versus 2 to 42 and
0 to 2 for \(^{123}\text{I-mIBG}\) (Supplemental Fig. 2).
DISCUSSION

First, we found that $^{18}$F-DOPA PET/CT was more sensitive than $^{123}$I-mIBG scan in detecting NB localizations in a well-selected population of NB children analysed at the time of first diagnosis, as proved by comparing PET/CT and $^{123}$I-mIBG SPECT/CT images in all patients. This is of particular interest, as in the two previous studies (17, 20) comparing $^{18}$F-DOPA and $^{123}$I-mIBG, SPECT/CT images were unavailable. In our study, $^{18}$F-DOPA PET/CT was even more sensitive in detecting soft tissue metastases and small bone/bone-marrow localizations.

Second, we found that $^{18}$F-DOPA PET/CT was a reliable diagnostic tool for evaluating treatment response after induction chemotherapy and provided important information on disease persistence. Specifically, $^{18}$F-DOPA PET/CT proved to be more sensitive than $^{123}$I-mIBG SPECT/CT in disclosing small and faint persistent bone and bone-marrow foci of pathological uptake after chemotherapy.

However, it would have been difficult to ascertain the real clinical impact of this finding if we had not tested the prognostic role of $^{18}$F-DOPA PET/CT. In other words, the real issue is whether the higher number of lesions disclosed by $^{18}$F-DOPA PET/CT after induction chemotherapy was associated to a higher probability of disease persistence/progression at the end of follow-up, or whether it was a simple and futile expression of diagnostic sensitivity. Accordingly, we evaluated the prognostic value of $^{18}$F-DOPA PET/CT both at the time of disease onset and after induction chemotherapy. To better understand each patient’s risk, we semi-quantified the disease burden by using the previously validated $^{18}$F-DOPA WBMB (18) and compared this with the mIBG WBS and the other recognized prognostic factors. Both scores measure the burden of the disease, excluding the primary tumour. So far, no reliable and validated methods have been used to quantify the extension and activity of the primary tumour on $^{123}$I-mIBG SPECT/CT (8,10) and on $^{18}$F-DOPA PET/CT images. In this regard, we found that, at the univariate level, only the disease stage on initial diagnosis (Stage 3 vs Stage 4) and the WBMB measured after induction chemotherapy (WBMB $>$ 7.5) were associated with prognosis. Notably, neither MYCN amplification nor mIBG score $>$3 after chemotherapy was significantly associated to progression-free survival. By contrast, a marked disease burden documented at the time of first diagnosis by means mIBG scan (mIBG WBS $>$ 46) and $^{18}$F-DOPA PET/CT (WBMB $\geq$45) showed an important trend towards significance ($p=0.06$ and $p=0.08$, respectively). This finding seems to be in line with what was reported in a recent paper by Lewington et al. (8), who showed that patients with a SIOPEN score
>48 at the time of first diagnosis had a significantly lower response rate to induction chemotherapy than patients with a SIOPEN score ≤48.

However, the Cox models showed that only an $^{18}$F-DOPA PET/CT WBMB score >7.5 measured after induction chemotherapy was associated with PFS. This cut-off had already been validated by our previous study [18], in which patients with WBMB >7.5 at the time of NB relapse had a higher probability of disease progression and death. In the present study, we found that, of the nine patients with negative mIBG WBS and persistent positive $^{18}$F-DOPA WBMB after induction chemotherapy, three had disease persistence/progression at the end of follow-up. All these three patients showed at least 4 persistent disease localizations on $^{18}$F-DOPA PET/CT, and in two the disease burden measured by means of WBMB was >7.5.

In addition, all 5 patients with $^{18}$F-DOPA WBMB >7.5 after induction therapy showed disease persistence/progression regardless their mIBG WBS value. From this point of view, $^{18}$F-DOPA WBMB was the most accurate parameter in stratifying the risk of HR-NBL patients after induction chemotherapy. We could speculate that a greater use of sensitive PET tracers, such as $^{18}$F-DOPA, could identify patients at very high risk in whom a more intense therapeutic regimen is warranted.

This study has some limitations: (1) its low statistical power (low number of patients and events); (2) the fact that the effect of different therapies performed after induction therapy were not considered in the prognostic analysis. In this context, we did not find any associations between the different risk factors and OS. Indeed, we used PFS alone as a surrogate of prognosis.

In addition, one Stage 4 mIBG negative patient was included in the study. SPECT/CT images were co-registered and fused by means of an appropriate software but were not acquired by means of a dedicated hybrid SPECT/CT system. This procedure is not optimal, as it does not provide the improved image quality yielded by CT attenuation correction. These issues may have caused the well-known diagnostic and prognostic power of the mIBG scan to be underestimated in this group of patients. In addition, the $^{18}$F-DOPA WBMB score is characterized by high variability, as a result of the high intrinsic sensitivity of PET/CT procedures and the possibility to express the extent of soft tissue metastases and uptake intensity; it therefore provides adequate information about patient risk, which may not be fully estimated by the mIBG score.
However, this is the first paper to prospectively analyse the diagnostic and prognostic role of $^{18}$F-DOPA PET/CT in a well-selected population of children (median age 28 months) affected by high- and intermediate-risk NB. Indeed, our preliminary data suggest that this tracer is more effective than $^{123}$I mIBG for the staging, treatment response evaluation and prognostication of NB patients.

In any case, to better evaluate the role of these two tracers in NB a direct comparison between $^{18}$F-DOPA and $^{124}$I-mIBG should be conducted. This analysis would eliminate any bias related to the different imaging procedures presented in this study.

Finally, although $^{123}$I-mIBG scan seems to be less sensitive than $^{18}$F-DOPA PET/CT, the principal advantage of mIBG remains its intrinsic theragnostic property. While considering that the pathological distribution of the tracers seems to be similar (17,18,28), there are not sufficient data to support the possibility of selecting patients for mIBG therapy by using $^{18}$F-DOPA PET/CT. From this point of view, DOPA cannot replace mIBG.

**CONCLUSION**

Our results confirmed good agreement between $^{18}$F-DOPA PET/ CT semi-quantification and $^{123}$I-mIBG scan in patients affected by NB at the time of first staging, in that a positive correlation between the two techniques was observed. However, $^{18}$F-DOPA PET/CT appears to be more sensitive than $^{123}$I-mIBG WBS with additional SPECT/CT to stage NB patients and, particularly, to evaluate disease persistence after induction chemotherapy. In time-to-event analyses, $^{18}$F-DOPA WBMB, evaluated after induction chemotherapy, remained the only risk factor independently and directly associated with disease progression. Further confirmation on a larger group of patient is required.

**COMPLIANCE with ETHICAL STANDARDS**

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**Conflict of interest:** The authors have nothing to disclose.
**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.

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

**KEY POINTS**

**Question:** is $^{18}$F DOPA PET/CT able to identify, after induction chemotherapy, NB patients with persistence of disease at higher risk of disease progression?

**Pertinent Findings:** In a clinical study including 18 NB patients at high and intermediate risk, $^{18}$F-DOPA PET/CT was a reliable diagnostic tool for evaluating treatment response after induction chemotherapy and provided important information on the presence of disease persistence. Specifically, $^{18}$F-DOPA PET/CT proved to be significantly more sensitive than $^{123}$I-mIBG SPECT/CT in disclosing small and faint persistent bone and bone marrow foci of pathological uptake after chemotherapy.

**Implication for patient care:** when compared with other prognostic factors and molecular imaging procedures, $^{18}$F-DOPA PET/CT performed after induction chemotherapy was able to better identify patients at very high risk amenable for further effective treatments.
REFERENCES


24. Fiebrich HB, Brouwers AH, Kerstens MN, et al. 6-[¹⁸F]-Fluoro-L-dihydroxyphenylalanine positron emission tomography is superior to conventional imaging with (123)I-metaiodobenzylguanidine scintigraphy, computer tomography, and magnetic resonance imaging in localizing tumors causing catecholamine excess. J Clin Endocrinol Metab. 2009;94:3922–30.


Fig. 1: 3-year-old child affected by stage 4 NB (MYCN amplified) with a primary thoracic localization. $^{18}$F-DOPA PET/CT (maximum intense projection and axial images, a and b) showed very intense uptake in the primary tumour and identified some bone and bone marrow metastases (arrows). $^{123}$I-mIBG WBS and additional SPECT/CT (axial images and anterior and posterior view, c, d and e) did not identify any site of pathological uptake.
Fig. 2: 3-year-old child affected by stage III NB (MYCN amplified) with a primary abdominal localization (not shown). Suspicious left paravertebral nodules detected on contrast-enhanced CT were negative on $^{123}$I-mIBG SPECT/CT (axial images a and b) but proved positive on $^{18}$F-DOPA PET/CT (axial images b and c).
Fig. 3: 4-year-old child affected by stage 4 NB (not MYCN amplified) with a primary abdominal localization.

At the time of first staging, both 18F-DOPA PET/CT and 123I-mIBG WBS with additional SPECT/CT well identified the primary tumour and diffuse bone/bone marrow metastases (a,c). After induction chemotherapy, both techniques identify some residual bone marrow metastases (b and d, black arrows) but 18F-DOPA PET/CT (maximum intense projection) revealed 3 small persistent bone marrow metastases (red arrows) not visualized by mIBG WBS.
Fig 4: Kaplan-Meier PFS curves according to: (a) $^{18}$F-DOPA WBMB $\leq 7.5$ and $>7.5$ measured after induction chemotherapy, (b) $^{18}$F-DOPA WBMB (median) $\leq 45$ and $>45$ measured before therapy, and (c) mIBG WBS score (3rd quartile) $\leq 46$ and $>46$ measured before therapy.
Fig 5: 2-year-old child affected by stage III NB (MYCN amplified) with a large calcific primary abdominal localization and major lymph-node involvement at the time of first staging. After induction chemotherapy, the large residual mass did not show any residual mIBG or DOPA uptake (axial image a, b and c). However, some calcific lymph-nodes negative on mIBG SPECT/CT were still evident on $^{18}$F-DOPA PET/CT (a, b and c, white arrows). The mIBG WBS calculated after induction chemotherapy was <3 (i.e. 0). By contrast, the WMBM was >7.5 (i.e. 16). By the end of follow-up (19 months) the patient had developed disease progression and died of disease.
Table 1: main clinical, histopathological and biochemical features of the patients (n=18).

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td><strong>Age</strong>, months</td>
<td>34 ± 19</td>
<td>(12-72)</td>
</tr>
<tr>
<td><strong>Sex</strong>, male</td>
<td>12 (67)</td>
<td></td>
</tr>
<tr>
<td><strong>Histopathology</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undifferentiated</td>
<td>6 (33)</td>
<td></td>
</tr>
<tr>
<td>Poorly differentiated</td>
<td>9 (50)</td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td>3 (17)</td>
<td></td>
</tr>
<tr>
<td><strong>Stage</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4 (22)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>14 (78)</td>
<td></td>
</tr>
<tr>
<td><strong>Treatment</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NB AR 01</td>
<td>15 (83)</td>
<td></td>
</tr>
<tr>
<td>LINES/Personalized</td>
<td>2/1 (17)</td>
<td></td>
</tr>
<tr>
<td><strong>MYC Amplification</strong>, n (%)</td>
<td>10 (56)</td>
<td></td>
</tr>
<tr>
<td><strong>Lactate dehydrogenase (LDH)</strong>, UI/L</td>
<td>3862 ± 3827 (573-16673)</td>
<td></td>
</tr>
<tr>
<td><strong>Homovanillic acid levels</strong>, umol/mmol creatinine</td>
<td>87.8 ± 105.0 (6.3-381)</td>
<td></td>
</tr>
<tr>
<td><strong>Vanilmandelic acid levels</strong>, umol/mmol creatinine</td>
<td>70.0 ± 82.2 (2.8-247)</td>
<td></td>
</tr>
</tbody>
</table>

**18F-DOPA WBMB score**

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<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>before induction chemotherapy</td>
<td>191.9 ± 349.1 (0-1120)</td>
<td></td>
</tr>
<tr>
<td>after induction chemotherapy</td>
<td>18.2 ± 43.1 (0-169)</td>
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</tr>
</tbody>
</table>

**123I-mIBG WBS score**

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<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>before induction chemotherapy</td>
<td>24.7 ± 20.9 (0-58)</td>
<td></td>
</tr>
<tr>
<td>after induction chemotherapy</td>
<td>3.6 ± 9.9 (0-42)</td>
<td></td>
</tr>
</tbody>
</table>

Descriptive statistics are mean ± standard deviation (range) for continuous data and n (%) for count data; abbreviations: WBMB = whole body metabolic burden; WBS = whole body scan.
Table 2. Patients based analysis. Detection rates (%) were calculated for each single diagnostic modality in each site of disease*.

<table>
<thead>
<tr>
<th></th>
<th>$^{123}$I-mIBG SPECT/CT</th>
<th>$^{18}$F-DOPA PET/CT</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before induction chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary Tumors</td>
<td>15 (83%)</td>
<td>17 (94%)</td>
<td>0.5</td>
</tr>
<tr>
<td>Soft Tissue metastases</td>
<td>6 (50%)</td>
<td>11 (92%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Bone/Bone marrow metastases</td>
<td>12 (92%)</td>
<td>13 (100%)</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>After induction chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary Tumor</td>
<td>13 (72%)</td>
<td>15 (83%)</td>
<td>0.6</td>
</tr>
<tr>
<td>Soft Tissue metastases</td>
<td>4 (33%)</td>
<td>9 (75%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Bone/Bone marrow metastases</td>
<td>5 (38%)</td>
<td>7 (54%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* NOTE: the denominator for detection rates on primary tumors is the total number of patients (n = 18), considering that they are all subjects with established cancer. For evaluations on soft tissue and bone/bone marrow lesions, the denominator is the maximum number of patients found to be positive by the two diagnostic modalities (n=12 for soft tissue; n=13 for bone/bone marrow lesions).

**= Exact McNemar significance probability.
Table 3. Lesion based analysis. Detection rates (%) were calculated for each single diagnostic modality in each site of disease*.  

<table>
<thead>
<tr>
<th></th>
<th>$^{125}$I-mIBG SPECT/CT</th>
<th>$^{18}$F-DOPA PET/CT</th>
<th>p**</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before induction chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft Tissue metastases</td>
<td>20 (41%)</td>
<td>42 (86%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bone/Bone marrow metastases</td>
<td>494 (93%)</td>
<td>522 (99%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>After induction chemotherapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soft Tissue metastases</td>
<td>11 (28%)</td>
<td>30 (77%)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Bone/Bone marrow metastases</td>
<td>54 (69%)</td>
<td>67 (86%)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

* NOTE: the denominator for detection rates is the maximum number of lesions found to be positive by the two diagnostic modalities: n=49 and n=39, before and after induction chemotherapy respectively, for soft tissue; n=529 and n=78, before and after induction chemotherapy respectively, for bone/bone marrow lesions. **= Exact McNemar significance probability.
Supplemental Fig 1: the diagram shows the mIBG WBS score and WBMB measured for each patient, before and after induction chemotherapy, according to the cut-off identified in the univariate analysis (colours indicate the different groups). The outcome is also specified at the bottom.
Supplemental Fig 2: Box plots of $^{18}$F-DOPA WBMB and $^{123}$I-mIBG WBS distributions. The bottom and top of the boxes are the 1st and 3rd quartiles, and the band inside the box is the median (2nd quartile). The whiskers represent the lowest value still within 1.5 interquartile range (IQR) of the lower quartile, and the highest value still within 1.5 IQR of the upper quartile. The inter-patient variability of $^{18}$F-DOPA scores proved higher than that of the $^{123}$I-mIBG scan scoring system.