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Discordance between Histopathological grading and Dual Tracer PET-CT findings (^{68}Ga-DOTATATE and FDG) in metastatic Neuroendocrine Neoplasms and outcome of ^{177}Lu-DOTATATE PRRT: does in-vivo molecular PET imaging perform better from 'prediction of tumour biology' viewpoint?

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Running Title: Histopathology vs. Dual tracer PET-CT in Metastatic NENs

Abstract:

Background and Aim: Discordance between histopathological grading and dual tracer PET-CT (^{68}Ga-DOTATATE and FDG) findings in neuroendocrine tumours (NETs), though not typical, can be encountered in real-world scenario. The aim of this study was to assess patients with discordance between WHO 2017 grade predicted molecular PET-CT imaging and the actual dual tracer PET-CT findings (by exploring their histopathological, immunohistochemical and molecular imaging characteristics), with a view to identifying the prognostic determinants effecting outcome in a peptide receptor radionuclide therapy (PRRT) set-up.

Methods: Thirty six patients of histopathologically proven inoperable, locally advanced/metastatic NETs, referred for PRRT were included in this study. The cohort was divided into two broad population groups: (a) those with discordance (between WHO 2017 grade predicted molecular imaging and the dual tracer PET-CT findings) and (b) control (showing both FDG and ^{68}Ga-DOTATATE uptake). The cohort was divided based on dual tracer PET-CT into: (i) metabolically FDG non-avid and SSTR expressing tumors, (ii) metabolically active and non-^{68}Ga-DOTATATE concentrating (SSTR expressing) and (iii) matched imaging characteristics with WHO 2017 grading system (showing both FDG and ^{68}Ga-DOTATATE concentrating disease) for statistical analysis. Statistical analyses were done on SPSS 23.0. Descriptive statistics was used to analyze categorical data, multivariate analysis was used to assess the correlation between different variables with progression free survival (PFS) and overall survival (OS). Kaplan-Meier was used for survival analysis to calculate median survival and to analyze the survival based on WHO 2017 grading and dual tracer PET. Cox proportional hazards regression analysis was used to determine predictors of survival (OS and PFS).

Results: In the entire cohort (n=36), 24 patients (66.7%) showed discordance whereas 12 patients (33.3%) were in the control group. Among the patients showing discordance: 14 patients (38.9%) had metabolically inactive and SSTR expressing disease and remaining 10 patients (27.8%) had FDG concentrating and SSTR non-expressing disease. Those in the control group, 12 patients (33.3%) had intermediate grade NETs and showed matched (^{68}Ga-DOTATATE and FDG concentrating lesions) disease. Multivariate analysis in patients with discordant findings demonstrated significant correlation of dual tracer PET with overall survival while no significant correlation could be established between WHO grade and overall survival in the discordant subgroups. No significant correlation could be appreciated between PFS and either dual tracer PET or WHO grading. The Kaplan-Meier survival analysis and Cox proportional hazards regression analysis demonstrated dual tracer PET-CT imaging to be significant prognostic determinant and predictor of outcome respectively.

Conclusion: In summary, in NET patients with discordance between the two parameters, dual tracer PET-CT with FDG and ^{68}Ga-DOTATATE performed better than WHO grading, differentiation status and immunohistochemistry in prognosticating and predicting outcome.

Introduction:

Neuroendocrine neoplasms (NEN) are heterogeneous group of widely distributed tumours comprising of both 'neural' and 'endocrine' components (*1*). The 'neural' component is based on identification of dense core granules (DGCs) and 'endocrine' component refers to synthesis and secretion of monoamines. Histopathological grading is supposed to be the most important prognostic factor so far, and helps in devising tailored therapeutic strategy for the patients. However, confusion and enigma has always surround this approach as, outliers are quite noticeable in day-to-day scenario.

Controversy has ever since surrounded the entity as early as, when the term 'carcinoid' (carcinoma like) (*2*) was introduced by Siegfried Oberndorfer in the start of 20th century, due to benign behaviour of small bowel tumours composed of argentaffin positive agyrophilic cells (*3*). This was criticised because of terminological confusion and diagnostic irregularities and was regarded misnomer, as these tumours displayed varying degrees of malignant potential (*4-6*). Later a plethora of terms referring to NETs were used viz. APUDoma, argentaffinoma, enteroendocrine tumours, tumours of diffuse endocrine system, agyrophilic cell carcinoma, etc (*7*). Gosset and Masson (1928) characterised carcinoids as NETs on basis of amine uptake and decarboxylation properties (*8*) whereas Williams and Sandler (1963) classified them according to embryonic divisions of digestive tract (*5*) and Arrigoni et al (1972) gave the concept of 'typical' and 'atypical' based on histopathological characteristics (*9*). In 1980, the World Health organisation (WHO) applied the term 'carcinoid' to describe all NETs except pulmonary NETs (*10*). However this led to more disaccord between pathologists and clinicians (*11,12*) whereas the Travis-WHO classification (1999) divided pulmonary and thymic NETs into typical carcinoid, atypical carcinoid and large cell (LCNEC) & small cell (SCNEC) neuroendocrine carcinomas (*13,14*). WHO, in 2000 and 2004 revised gastroenteropancreatic (GEP) and pulmonary/mediastinal NETs based on differentiation and mitotic index & necrosis respectively (*15, 16*). The WHO 2010 classification re-defined the entire group of tumours as Neuroendocrine neoplasms (NENs) and subdivided them according to proliferative index (Ki-67/Mib-1) and mitotic counts (*17, 18*).

Ambiguity of 2010 WHO Grading System and need for revision: The 2010 WHO classification categorized NENs into three grades; grade 1 and 2 being well differentiated NET while grade 3 referred to poorly differentiated neuroendocrine carcinoma (NEC) (*17,18*). In general, a well

differentiated NEN is composed of cells showing minimal to moderate atypia, lacks necrosis and expresses general markers of neuroendocrine differentiation (diffuse and intense synaptophysin and chromogranin A) whereas poorly differentiated NEN is composed of highly atypical small or large cells expressing faint neuroendocrine differentiation markers. In case of discordance between differentiation and proliferative index or when tumours do not concur with the predicted course, the National Comprehensive Cancer Network (NCCN) recommends that clinical judgement should trump the grading system (*19*). In case of discrepancy between the proliferative and mitotic indices, the higher grade should prevail.

The 2010 WHO grading system was flawed in addressing the contrast between 'grade' and 'differentiation'. While 'grade' refers to the aggressiveness of tumour cells in terms of their potential for rapid growth and spread, 'differentiation' is the morphologic resemblance of tumour cells to islets of Langerhans (*20, 21*). Hence, it was possible that well differentiated NETs could be technically graded as grade 3 (G3), but may not be sensitive to chemotherapy regimen used in poorly differentiated NECs (G3 NEC) (*21*). These well differentiated NETs which are technically classified as grade 3 NEC (based on proliferation index, WHO 2010) may not be sensitive to chemotherapy regimen indicated for grade 3 NECs. Interestingly, if an adequate number of pathological specimens are available for an accurate mitotic count, most grade 3 NETs contain a proportion of cells with a mitotic rate fewer than 20 per 10 high power field (hpf) and still lower grade regions may be present elsewhere in the tumour focus (*20*), hence rendering proliferation index and mitotic counts to be focal, rather reflective of overall tumour composition. Furthermore, the genomic composition of grade 3 NET resembles those of low grade NET, i.e., MEN1, DAXX and ATRX mutation, and differ distinctly from that of poorly differentiated NEC, i.e., p53 and RB1 mutation (*22*). All these led to revised WHO classification of NETs in 2017, which along with its comparison to 2010 WHO classification system is detailed in table 1 (*23*).

Furthermore, studies evaluating dual tracer PET using FDG (flurodeoxyglucose) and ^{68}Ga-DOTATATE (DOTA Tyrosine^{3} Octreotate), demonstrated relatively lesser FDG concentration than ^{68}Ga-DOTATATE in patients of grade 3 NEC, as against theoretically anticipated (G3 NEC, WHO 2010) (*24*). Receptor targeted molecular imaging with 'dual-tracer' positron emission tomography-computed tomography (PET CT) scans using FDG and ^{68}Ga-DOTATATE provides

overall, semiquantitative assessment of tumour biology and burden. Hence, it may potentially score over current conventional classification and grading systems, that mainly relies on focal needle sampling of the most accessible lesion (primary and/or metastatic) in order to guide management strategy. The present study tries to evaluate the plausibility of the above mentioned concept.

Materials & Methods:

A total of thirty-six (n=36) patients with histopathologically proven NET, who had undergone peptide receptor radionuclide therapy (PRRT) at our centre, were retrospectively included and their records were analyzed. This retrospective study was approved by our institutional scientific and medical ethics committee and the requirement to obtain informed consent was waived, as these patients were referred for PRRT and the FDG and ^{68}Ga-DOTATATE scans were done as a part of routine pre-therapy workup. The patients were categorized based on current 2017 WHO classification. The cohort was divided into two broad groups: (a) with discordance (between WHO 2017 grade predicted dual tracer PET-CT findings and the actual dual tracer PET-CT findings) and (b) control (showing both FDG and ^{68}Ga-DOTATATE uptake). The cohort was divided based on dual tracer PET into: (i) metabolically FDG non-avid and SSTR expressing, (ii) metabolically active FDG avid and SSTR non-expressing and (iii) matched (showing both metabolic activity and SSTR expression) and according to WHO 2017 grading system for statistical analysis. SUVmax of 2.5 on FDG PET CT was standardized to SUVmax of 9.0 on ^{68}Ga-DOTATATE PET-CT. Inclusion criteria: (a) histopathologically proven neuroendocrine tumour/carcinoma (b) discordance between histopathological (WHO 2017) grade predicted dual tracer PET and actual dual tracer PET findings.

Statistical analysis:

All statistical analyses were performed by SPSS software, version 23.0 (SPSS Inc., Chicago, USA). Descriptive statistics was used to analyse categorical data. Multivariate analysis was used to evaluate the correlation between different variables with progression free survival (PFS) and overall survival (OS). Kaplan-Meier product limit method was used for survival analysis to calculate median survival and to analyse the survival based on WHO 2017 grade and dual tracer

PET. The variables: dual tracer PET and WHO 2017 grade determining OS and PFS were compared using Log-rank test. Cox proportional hazards regression analysis was used to identify predictors of outcome (OS and PFS). Patients who were alive or with non-progressed disease (for OS and PFS respectively) at the time of analysis or last contact were censored. A two-tailed p-value <0.05 was considered statistically significant and the hazards ratio (HR) presented with 95% confidence interval (CI).

Results:

Thirty six patients (n=36), 24 males (66.7%) and 12 females (33.3%) of histopathologically proven NET were identified and retrospectively analysed. The median age for the cohort was 50 years (minimum 25 and maximum 66 years). The referral for PRRT was made due to metastatic and/or inoperable locally advanced disease progressing on prior therapy (octreotide therapy or chemotherapy). Table 2 illustrates an overview of patient demographics.

Pancreas was the most commonly involved primary site with 12 patients (33.3%) followed by unknown primary (7 patients, 19.4%), rectum (5 patients, 13.9%), small bowel (4 patients, 11.1%), lungs (3 patients, 8.3%), mediastinum (2 patients, 5.6%) and stomach, gall bladder and skin appendages (Merkel cell carcinoma), each with a single patient (2.8%). According to 2017 WHO grading system, 41.7% (15 patients) were G2 NET, followed by 7 patients (19.4%) each in G1 NET, G3 NET and G3 NEC respectively. In the cohort, 24 patients (66.7%) showed discordance whereas 12 patients (33.3%) were in the control group. Among the patients showing discordance: 14 patients (38.9%) had metabolically inactive and SSTR expressing disease and remaining 10 patients (27.8%) had metabolically active and SSTR non-expressing disease. Those in the control group, 12 patients (33.3%) and intermediate grade NETs and showed matched (metabolically active and SSTR expressing) disease.

A total of 27 patients (75% of the patients, n=27) had well differentiated histology, 19.4% (n=7) had poorly differentiated histology and in 2 patients (5.6%), the histology was not available. 30 patients (83.3%) were synaptophysin positive and in remaining 6 patients (16.7%) the data was unavailable. Chromogranin A was found positive in 26 patients (72.2%), negative in 3 patients

(8.3%) and in remaining 7 patients (19.4%), the relevant data was unavailable. However, no definitive pattern could be established in chromogranin A negative patients. Similarly, no definitive trend or pattern was appreciated between epithelial & other IHC markers and other variables, could be mainly in part due to inconsistent selection of IHC markers in patients and hence lack of uniformity (Table 3).

Of the twenty four (n=24) patients with discordant NET (in terms of WHO grade predicted and actual dual tracer PET findings): 7 (~ 30%) patients progressed [2 out of 14 patients (14.3%) with metabolically inactive and SSTR expressing and 5 out of 10 patients (50%) with metabolically active and SSTR non-expressing] and 8 patients (~ 33.3%) succumbed to the disease [1 out of 14 patient (7.1%) with metabolically inactive and SSTR expressing and 7 out of 10 patients (70%) with metabolically active and SSTR non-expressing]. Among the control group of twelve (n=12) patients with matched disease 3 patients (25%) progressed and 5 patients (41.7%) died. In the entire cohort, the median cumulative progression free survival for the cohort was 83 months (82.9 months for metabolically inactive and SSTR expressing and 49.8 months for metabolically active and SSTR non-expressing) and overall survival was 118 months (90 months for metabolically inactive and SSTR expressing and 61.2 months for metabolically active and SSTR non-expressing). Categorization based on WHO 2017 grading did not yield such trends and results (figure 1 & 2). The dual tracer PET-CT characteristics of the patient population has been detailed in table 4.

On multivariate analysis, the only significant correlation was between dual tracer PET and overall survival (p=0.01), however no significant correlation was flagged between any of the variables and progression free survival in this study.

The determinants: dual tracer PET and WHO 2017 grading compared by Kaplan-Meier analysis and KM plots were generated for PFS and OS (figure 1 & 2). Significant difference was noticed between the KM plots when categorization was done on the basis of dual tracer PET (p=0.05 for PFS and p=0.02 for OS; Log Rank test) as against when the cohort was categorised on the basis of WHO 2017 grading system (p=0.39 for PFS and 0.67 for OS; Log Rank test) and analysed. Cox proportional hazards regression analysis was employed to analyze dual tracer PET vs WHO 2017 grading system as predictor of outcome (PFS and OS) and showed dual racer imaging as

independent predictive prognostic variable (HR 0.23, 95% CI: 0.31 - 1.67, p=0.03 for PFS and HR 0.027, 95% CI: 0.002 - 0.35, p=0.005 for OS). No significant statistics could be achieved for WHO 2017 grading system (HR 0.49, 95% CI: 0.061 - 3.861, p=0.5 for PFS and HR 0.301, 95% CI: 0.3 - 3.013, p=0.31 for OS).

A smaller sub-study was done, categorising patients based on ^{68}Ga-DOTATATE uptake (Krenning score). Two patients (n=2) belonging to Krenning score 1 were denied PRRT. Of 5 patients (n=5) of Krenning score 2: 2 patients received single cycle of PRRT and the remaining 3 patients received 2 to 3 cycles of PRRT respectively (first cycle was administered mainly on trial and/or compassionate grounds due to paucity of other available alternatives). Additional PRRT cycles in Krenning 2 patients were administered either due to some initial symptomatic benefit or as part of hitherto combined chemo-PRRT trial, which in almost all cases after third cycle of PRRT showed disease progression and further PRRT was withheld. Of 7 patients belonging to Krenning score 1 and 2, 5 patients (71.4%) progressed and all 7 patients (100%) succumbed to disease with adverse clinical outcome (marked by relatively brief OS and PFS). The cohort with Krenning score 3 and 4 comprised 29 patients (80.6%); 10 patients (27.8%) and 19 patients (52.8%) belonging to Krenning score 3 and 4 respectively. Of these 29 patients, 5 patients (17.2%) progressed and 6 patients (20.7%) died. Hence higher SSTR expression was associated with favourable outcome and vice versa.

Discussion:

The WHO 2010 grading system was revised in 2017 to identify well differentiated NET with Ki67 > 20% and poorly differentiated NEC with Ki67 > 20% (Earlier in 2010 grading, all NETs with Ki67 > 20% were considered NEC). Ideally Grade I NETs should have high ^{68}Ga-DOTATATE uptake and low FDG uptake and Grade III NETs and NECs should have low ^{68}Ga-DOTATATE and high FDG uptake. But in our clinical experience we found obvious outliers where there were high FDG and low ^{68}Ga-DOTATATE uptake in Grade I NETs and vice versa (high ^{68}Ga-DOTATATE and low FDG in Grade III NETs and NECs). Grade II NETs showed mixed uptake. Most of the times, histopathological grading serves as an excellent prognostic marker and in most cases the functional imaging findings are in concordance with it. But in situations where there is

discordance, histopathological grading may not be reflective of the exact and overall tumour biology as clinically observed and affirmed by this study.

Here we specifically evaluated the NETs showing discordance between actual functional imaging findings (of ^{68}Ga-DOTATATE and FDG) and 2017 WHO grade predicted imaging findings. These entities albeit not regularly encountered in normal clinical scenario, do practically exist. The study group comprised of patients with contradictory imaging findings (eg. ^{68}Ga-DOTATATE negative and FDG positive scan in well differentiated tumours, Grade I and vice versa). We tried to evaluate and explain this paradoxical behaviour of some of the NETs and whether dual tracer PET-CT (using ^{68}Ga-DOTATATE and FDG) can aid in therapeutic decision making and predict the outcome to treatment, especially PRRT. The main objective of this study was to evaluate the validity of dual tracer PET-CT (functional imaging using FDG and ^{68}Ga-DOTATATE) as prognostic marker in comparison to other available determinants (eg histopathology) especially in deciding PRRT as therapeutic option and in predicting outcome to PRRT. This concept resonated with the WHO's approach in classifying grade 3 NETs into well differentiated (G3 NET) and poorly differentiated (G3 NEC), exhibiting stark contrast in terms of their biological behaviour and response to treatment (particularly chemotherapy) and ultimately culminating in the current 2017 WHO NET grading system.

This nuance form the usual and predicted course may be attributable secondary to (a) high-grade transformation of original low grade disease, and (b) overestimation and generalisation of histopathological and IHC findings to be representative of the tumour and/or the overall disease burden; which essentially is, only a localised finding 'focal' in its representation to the extent of sampling needle tip or the tissue specimen biopsied, in most, if not all the cases. Vis a vis discordant NETs, the current database of available manuscripts are relatively deficient with only occasional reports which are both nascent and ambiguous in their understanding of the entity. Tang et al (2016), in their study of histopathological, IHC and genetic constitution of well differentiated NETs, deduced that 'mixed grades' do exist within the population of well differentiated NETs and are distinguishable from poorly differentiated NECs by their unique phenotype, proliferative indices and genotype, either at the time of diagnosis or afterwards at both primary and metastatic sites (*25*). Nuñez-Valdovinos et al (2018), in a large Spanish tumour registry study, inferred that

substantial clinical heterogeneity is observed for both G2 and G3 NENs and analysis of large national database (RGETNE) suggested that tumour morphology is a valuable aid in addition to proliferation index, to further stratify clinical outcome and prognosis in patients with gastro-entero-pancreatic NENs (GEP-NENs) (*26*). Choe et al (2018) in their review article (*22*) highlighted that functional imaging specifically somatostatin receptor scintigraphy (SRS with ^{68}Ga-DOTATATE) and FDG may be helpful in distinguishing well differentiated NETs from poorly differentiated NECs (*27*); especially in challenging situations where there is a discrepancy between imaging features and histology. Particularly in context of NECs, considering the fact that they do not always show positive IHC markers (*28*) and/or in cases where tissue sample may not be representative of the entire tumour and/or disease burden, functional imaging, particularly 'dual tracer' PET has an important role to play (*29*). Basu et al (2015) also concluded that even in presence of different proliferative indices, inverse correlation in tracer uptake highlighted by 'dual tracer' PET scans with ^{68}Ga-DOTATATE and FDG are propitious as: (a) in-vivo depiction of overall tumour phenotype resulting from multiple putative and unknown interactions at cellular level; (b) in cases of inter-lesional and intra-lesional heterogeneity rendering histopathology and IHC to possible sampling error and under-representation; and (c) in assessing tumour biology at intermediate grading indices (*24*). Thapa et al (2016) and Zhang J et al (2020) in their studies showed that high FDG uptake was associated with poorer outcomes in NETs treated with PRRT (*30, 31*). However, symptomatic improvement was observed in most cases irrespective of grade and FDG uptake, high pre-therapy FDG uptake in both low-grade and high-grade NETs predicted an inferior outcome and was associated with disease progression. Although these studies emphasize the prognostic implication of FDG uptake, the first study by Thapa et al employed WHO 2010 NET grading system and did not take into account, the value of 'dual tracer PET (combined results of FDG & ^{68}Ga-DOTATATE scans)'; whereas both did not evaluate the discordance between actual functional imaging findings and histopathological grade predicted functional dual tracer PET findings. It is imperative from the literature data, that both FDG and ^{68}Ga-DOTATATE uptake would form determinants of response, and their relative concentration on PET-CT imaging would be an important molecular imaging parameters for such prediction (*32-35*). In a previously published study from our centre by Sampathirao et al (2017) investigated the potential role of dual tracer PET-CT (with FDG & ^{68}Ga-DOTATATE) in detection of primary in carcinoma of unknown primary (CUP-NETs), and the findings on PET-CT usually correlated well

with tumour proliferation index, however few outliers were noticed (*36*). A few of these outliers may have been included in the present study, that looked primarily into their outcome viewpoint.

For such clinical situations, functional imaging using dual tracer (^{68}Ga-DOTATATE & FDG) proved useful as individual sampling of all the lesions will be practically impossible for obvious practical and ethical reasons. 'Dual tracer' functional imaging with ^{68}Ga-DOTATATE and FDG seems potentially advantageous and pragmatic due to: (a) non interventional representation of whole body disease burden; (b) relative tracer uptake reflects differentiation status and aggressiveness of the lesions; (c) can direct appropriate treatment strategy; (d) effective response evaluation and prognostication; and (e) to a lesser extent, can guide towards diagnosis (figures 3, and 4). The present study is unique in the way that it evaluated a very small and specified entity: discordance between WHO 2017 grade predicted dual tracer PET-CT findings and the actual dual tracer PET-CT findings. Encouraging results were noted supporting the role of 'dual tracer' functional imaging in solving the conundrum surrounding the management and prognosis, and is imperative in its concept and approach. In our study, the outcome (progression free and overall survival) of the patients with discordance more closely correlated with dual tracer PET findings (FDG and ^{68}Ga-DOTATATE PET-CT) as against 2017 WHO grading system. Furthermore, dual tracer PET (as against 2017 WHO grading system) was found to be independent prognostic factor for progression free and overall survival.

The major limitation of the study was its retrospective design contributing inherently as well as due to lack of homogeneous protocol in histopathology (especially with respect to IHC markers) and tumour marker evaluation, due largely to lack of standardized approach among referring institutions and hospitals. Another possible contributor worth mentioning was non-uniformity among the cohort with respect to disease burden and general condition, which could affect the duration of OS and PFS in these heavily pretreated patients; as patients were referred for PRRT at various stages in the course of disease. The fact that genetic mutations and pathways were not studied could present as one of the major pitfall and, which in our opinion could be pivotal to the phenomenon of discordance. This once understood, could potentially be a 'game-changer' leading to paradigm shift in our present day understanding and hence, management of discordant NETs. However, this study did present some ostensible salient learnings: (a) novel concept of

discordance between WHO 2017 grade predicted molecular imaging and the actual dual tracer PET-CT findings were evaluated and demonstrated encouraging results in favour of 'dual tracer' PET; (b) highlighted the possible pitfalls with histopathological grading and its reliability in devising personalised treatment strategy; and (c) need of a well-structured prospective study recruiting homogeneous patient cohort and encompassing all the possible determinants including genomic and proteomic analysis, is the need of the hour in deciphering this medical conundrum.

Conclusion:

Dual tracer PET (using FDG and ^{68}Ga-DOTATATE) is a promising entity in NET management and may perform better than histopathology in evaluating overall tumour burden and biology especially in clinical decision making and selecting patients who will benefit from PRRT. The findings and deliberations in the present work indicate that histological classification alone is not sufficient - a focal high MIB-1 index should not preclude a patient from PRRT (if the somatostatin receptor PET imaging reveals a high receptor expression) and on the other hand, a low tumor proliferation rate at the time of first diagnosis does not unarguably predict concordant biology in all the lesions, and it is possible to encounter a probable temporal change in the tumor grade (de-differentiation), hence a multifaceted consideration including the dual tracer PET-CT features would be greatly useful and adds scientific basis to the management strategy. Discordance in neuroendocrine tumours could be multifaceted and more complex, hence continued multi-disciplinary approach and investigation would be the key to a more detailed insight and understanding of these 'zebras' of the oncological practice.

KEY POINTS:

-- Question:

This study intended to address the issue of 'Discordance between histopathological grading and dual tracer PET-CT (^{68}Ga-DOTATATE and FDG) findings in metastatic NENs', a possible intriguing scenario in routine clinical practice.

-- Pertinent Findings:

The present retrospective observational study primarily examined the cohorts with discordance (between WHO 2017 grade predicted molecular imaging and the dual tracer PET-CT findings) and the outcome of PRRT. The results demonstrated that dual tracer PET-CT imaging to be a significant prognostic determinant and predictor of outcome.

-- Implications for Patient Care:

The findings in the present work indicate that histological classification alone is not sufficient - a focal high MIB-1 index should not preclude a patient from PRRT (if the somatostatin receptor based PET reveals a high receptor expression) and on the other hand, a low tumor proliferation rate at the time of first diagnosis does not unarguably predict concordant biology in all the lesions, and it is possible to encounter a probable temporal change in the tumor grade (de-differentiation), hence a multifaceted consideration encompassing the dual tracer PET-CT features along with histopathology would be greatly useful and adds scientific basis to the management strategy.

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Figure 1: Kaplan-Meier curves (a) and Cox Proportional Hazards Survival curves (b) for PFS: a (i) KM curves for PFS on the basis of dual tracer PET, a (ii) KM curves for PFS on the basis of 2017 WHO grading system; b (i) Cox Proportional Hazards Survival curves for PFS on the basis of dual tracer PET & b (ii) Cox Proportional Hazards Survival curves for PFS on the basis of 2017 WHO grading system. KM and Cox Proportional Hazards Survival curves demonstrated significantly better progression free survival for metabolically inactive and SSTR expressing group than metabolically active and SSTR non-expressing group when cohort was analyzed on the basis of dual tracer PET. Analysis based on 2017 WHO grading system did not yield any significant difference.

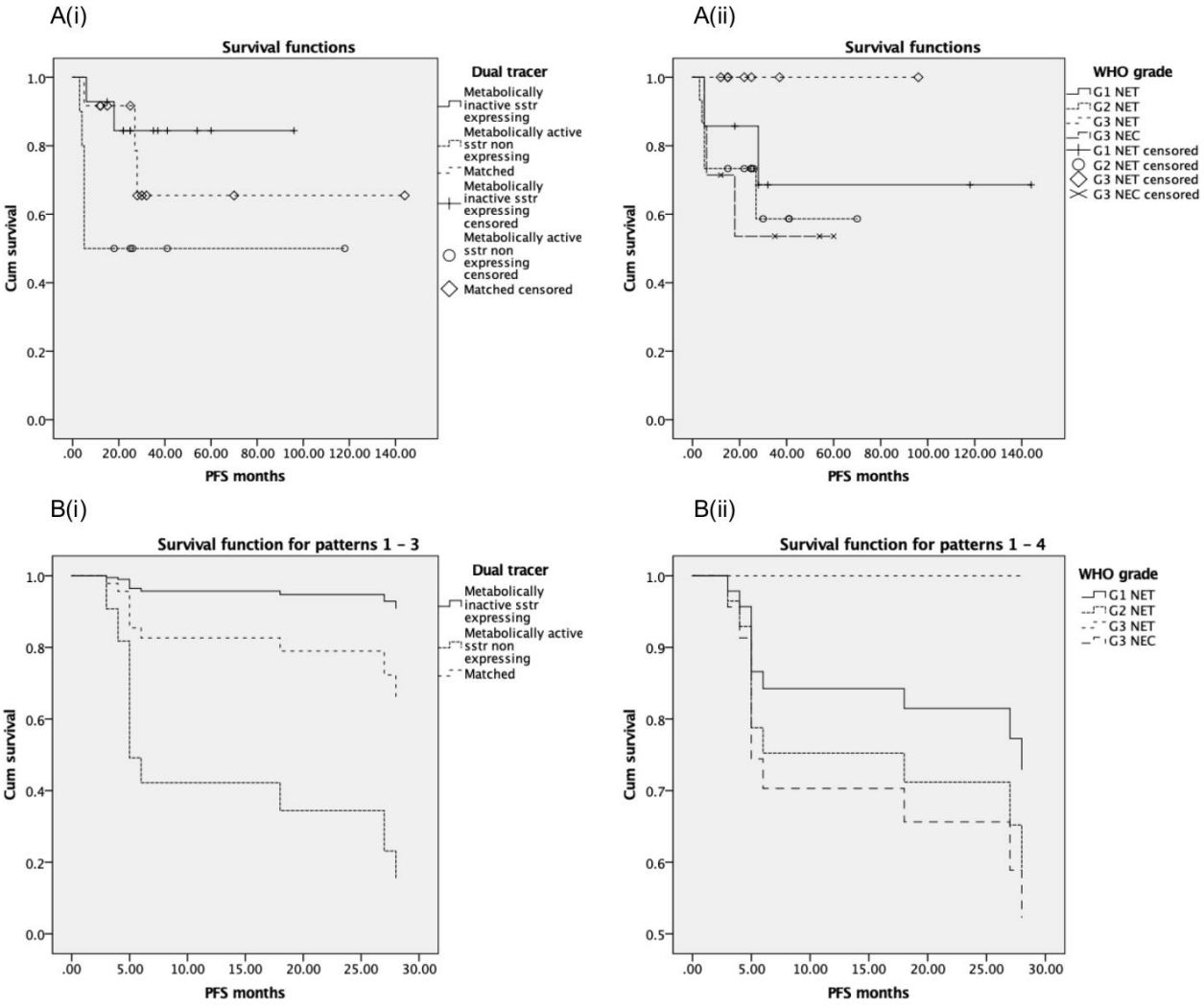


Figure 2: Kaplan-Meier curves (a) and Cox Proportional Hazards Survival curves (b) for OS: a (i) KM curves for OS on the basis of dual tracer PET, a (ii) KM curves for OS on the basis of 2017 WHO grading system; b (i) Cox Proportional Hazards Survival curves for OS on the basis of dual tracer PET & b (ii) Cox Proportional Hazards Survival curves for OS on the basis of 2017 WHO grading system. KM and Cox Proportional Hazards Survival curves demonstrated significantly better overall survival for metabolically inactive and SSTR expressing group than metabolically active and SSTR non-expressing group when cohort was analyzed on the basis of dual tracer PET. Analysis based on 2017 WHO grading system did not yield any significant difference.

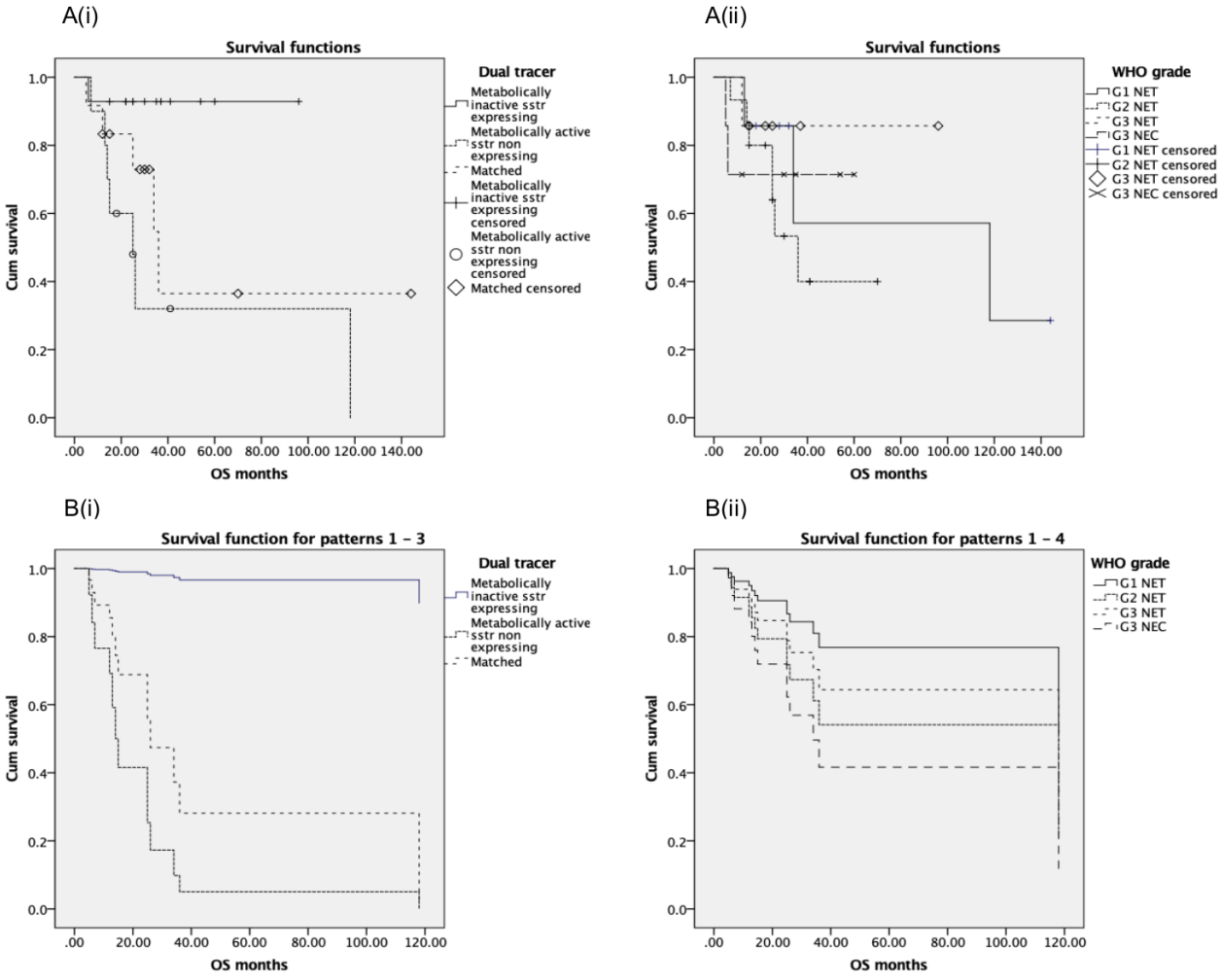


Figure 3: 61 years old male, case of metastatic NET to liver, mediastinal and abdominal nodes and multiple skeletal sites with unknown primary. Histopathology revealed poorly differentiated neuroendocrine carcinoma, with synaptophysin, chromogranin and CK19 positive on IHC. Despite high proliferative index of 25%, ^{68}Ga-DOTATATE PET-CT at baseline revealed intense SSTR expression in hepatic and skeletal lesions and mediastinal, abdominal and pelvic nodes whereas FDG PET-CT showed a single metabolically active para-celiac node. Follow up ^{68}Ga-DOTATATE PET CT revealed partial response with decrease in size and SSTR expression in almost all the lesions whereas FDG PET CT did not show any abnormal uptake, suggesting complete metabolic resolution. Despite poorly differentiated grade 3 neuroendocrine carcinoma (G3 NEC, WHO 2017), the dual tracer PET CT studies suggested favourable tumour biology which was adequately clinically translated. Post 3# PRRT the patient is doing fine with significant symptomatic and morphological improvement.

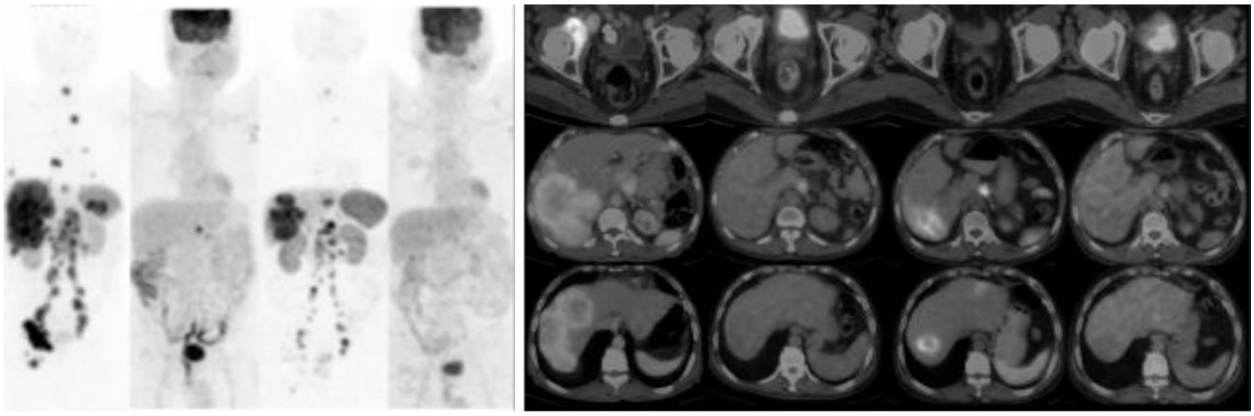


Figure 4: 64 years old lady, case of metastatic NET to liver and skeletal sites with unknown primary, presented with pain abdomen and weight loss and was referred for PRRT in view of SSTR expressing metastatic neuroendocrine tumour. Histopathology (of liver lesion) revealed metastatic NET, well differentiated, Mib 1 index 24% with synaptophysin & chromogranin positive and CDX2 negative on IHC. Baseline ^{68}Ga-DOTATATE PET CT revealed multiple areas of increased tracer uptake (SSTR expression) in both lobes of liver and skeletal sites with no abnormal hypermetabolism evident on baseline FDG PET-CT. Follow up dual tracer PET-CT scans, after 4# of PRRT demonstrated decrease in number of smaller hepatic metastases with mild interval decrease in size of the larger hepatic lesion in the left lobe, overall partial response. Here dual tracer PET-CT scans appeared to be in agreement with the histopathological finding of well differentiated grade 3 neuroendocrine tumour (G3 NET, WHO 2017) and the findings were adequately clinically translated.

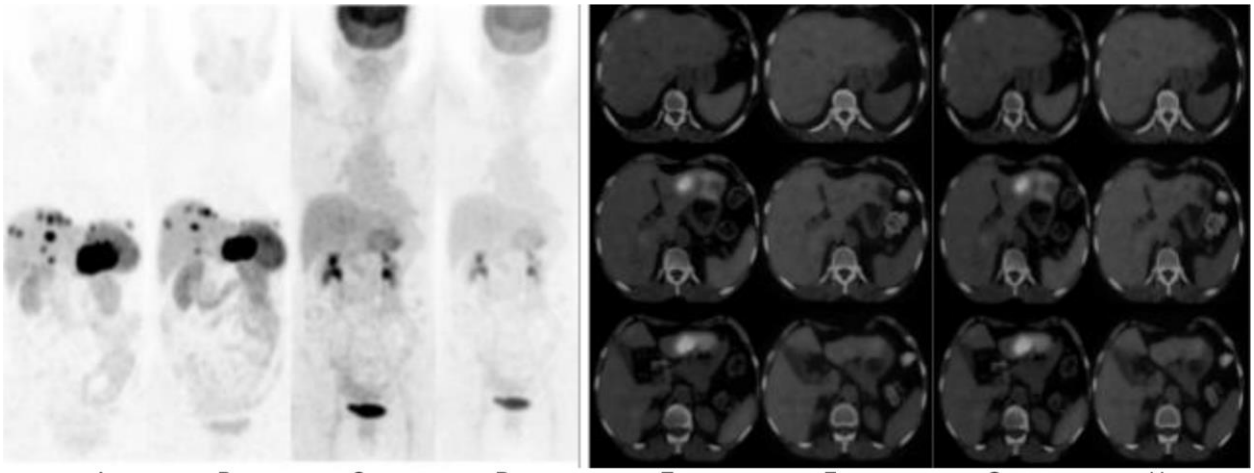


Table 1: WHO NET classification: 2010 vs. 2017

WHO 2010	Ki-67 Index	Mitoses/10 HPF	WHO 2017	Ki-67 Index	Mitoses/10 HPF
Well differentiated NENs			Well differentiated NENs		
NET grade I	<3	<2	NET grade 1	<3	<2
NET grade 2	3 to 20	2 to 20	NET grade 2	3 to 20	2 to 20
			NET grade 3	>20	>20
Poorly differentiated NENs			Poorly differentiated NENs		
NEC grade 3 (small cell or large cell)	>20	>20	NEC grade 3	>20	>20
			Small cell type		
			Large cell type		
MANEC			MiNEN		

Table 2: Patient demographics

Characteristics	Number (n) and percentage of patients
Total number of patients (n)	36
Male/Female	24/12
Median age/Range	50/25 - 66 (years)
Site of primary	
Pancreas	12 (33.3%)
Unknown	7 (19.4%)
Rectum	5 (13.9%)
Small bowel	4 (11.1%)
Lung	3 (8.3%)
Mediastinum	2 (5.6%)
Stomach	1 (2.8%)
Gall bladder	1 (2.8%)
Skin appendages (Merkel cell carcinoma)	1 (2.8%)
WHO grade (2017 classification)	
G1 NET	7 (19.4%)
G2 NET	15 (41.7%)
G3 NET	7 (19.4%)
G3 NEC	7 (19.4%)
Differentiation status	
Well differentiated	27 (75.0%)
Poorly differentiated	7 (19.4%)
Not known	2 (5.6%)

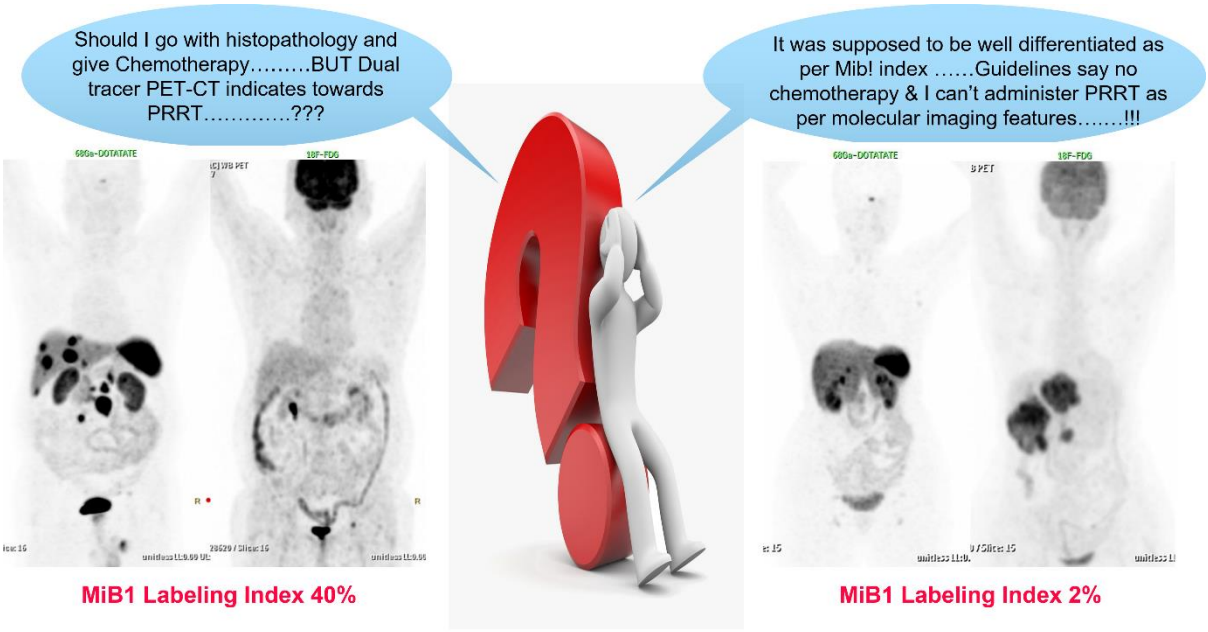
Table 3: Histopathological characteristics of the patient population

Synaptophysin (IHC)	
Positive	30 (83.3%)
Negative	6 (16.7%)
Chromogranin A (IHC)	
Positive	26 (72.2%)
Negative	3 (8.3%)
Not known	7 (19.4%)
Epithelial markers (AE1/AE3; IHC)	
Positive	11 (30.6%)
Negative	2 (5.6%)
Not known	23 (63.9%)
Other IHC markers (ATRX, Cytokeratin, CD56, CK7,19,20 & CDX2)	
Positive	10 (27.8%)
Not known	26 (72.2%)

Table 4: Dual tracer PET characteristics of the patient population

Baseline FDG uptake (SUVmax):	
<5	14 (38.9%)
5 to 10	5 (13.9%)
10 to 20	10 (27.8%)
> 20	7 (19.4%)
Baseline DOTATATE uptake	
Krenning 1	2 (5.6%)
Krenning 2	5 (13.9%)
Krenning 3	10 (27.8%)
Krenning 4	19 (52.8%)
Dual Tracer	
Metabolically inactive & SSTR expressing	14 (38.9%)
Metabolically active & SSTR non-expressing	10 (27.8%)
Matched (Metabolically active & SSTR expressing)	12 (33.3%)

Graphical Abstract:



With which regimen the Response and PFS & OS going to be longer???