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Clinical Radiology

Accuracy of Contemporary Oesophageal Cancer Lymph Node Staging with Radiological-Pathological Correlation

--Manuscript Draft--

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Abstract:	<p>Aim Accurate lymph node staging is vital to inform optimum treatment decisions in patients with oesophageal cancer. This study evaluates the accuracy of contemporary N-staging and provides radiological-pathological correlation in patients with lymph node metastases (LNMs) that were radiologically staged N0.</p> <p>Materials and Methods One hundred and twelve patients were included who underwent surgery alone (n=41) or had neo-adjuvant therapy (n=71) between October 2010 and December 2015. Contrast-enhanced CT (CECT), endoscopic ultrasound (EUS) and PET/CT N-stage were compared to pathological N-stage [node-negative (N0) vs node-positive (N+) groups]. Fifty LNMs from 15 patients pre-operatively staged as N0 were measured and the maximum size recorded.</p> <p>Results Accuracy, sensitivity and specificity of N0 vs N+ disease with CECT, EUS and PET/CT was 54.5%, 39.7% and 77.3%, 55.4%, 42.6% and 75.0%, and 57.1% 35.3% and 90.9%, respectively. All modalities were more likely to under-stage nodal disease; CECT (X2 32.890, df 1, p<0.001), EUS (X2 28.471, df 1, p<0.001) and PET/CT (X2 50.790, df 1, p<0.001). PET/CT was more likely to under-stage nodal disease than EUS (p=0.031). Median LNM size was 3 mm, with 41 (82%) of LNMs measuring <6 mm and 22 (44%) classified as micro-metastases (≤ 2 mm).</p> <p>Conclusion This study has demonstrated poor N-staging accuracy in the modern era of radiological staging. Eighty-two percent of LNMs measured <6mm, making direct identification extremely challenging on medical imaging. Future research should focus on investigating and developing alternative surrogate markers to predict the likelihood of</p>

LNMs.

Accuracy of Contemporary Oesophageal Cancer Lymph Node Staging with Radiological-Pathological Correlation

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Author Contributions

1 guarantor of integrity of the entire study	SAR
2 study concepts and design	KF SAR
3 literature research	KF AC
4 clinical studies	AC PF WGL SAR
5 experimental studies / data analysis	N/A
6 statistical analysis	KF
7 manuscript preparation	KF AC PF SAR
8 manuscript editing	KF AC PF WGL SAR

Dear Editor,

On behalf of the authors, I wish to thank you for accepting our manuscript for publication in Clinical Radiology.

Please find our responses to the reviewers' comments below.

Reviewer #1

Major comments: none

Minor comments:

Page 5, line 80: It would be worth describing here the criteria used to define positive nodes at CT.

A sentence describing the criteria used has been included.

Page 6, line 108: which criteria do you adopted to define positive nodes at PET?
Please state.

Our criteria for defining positive lymph nodes on PET have been included.

Figures: authors could add two figures showing 1) a true positive nodal case and 2) a false negative nodal case, each showing CT, ultrasound, PET and if possible pathology.

We have included a single figure (Figure 1) showing CT, PET, fused PET/CT images and the pathology slide for a 'false negative' case. After discussion, we decided not to include a 'true positive' case. The authors felt this would take up valuable space without adding much value because the general radiological community are likely to recognise a pathological lymph node.

Reviewer #2

1. the length of time between imaging and surgery/pathological assessment, which has an unknown impact on how severely the various imaging modalities understage. This is mentioned by the authors as a weakness and cannot be rectified; such is the nature of retrospective studies.

This limitation has not been changed.

2. The small proportion of pathological specimen available for retrospective analysis. This is not seem to be discussed by the authors as a weakness. It is a shame since the part of the manuscript dealing with size measurement of the proven metastases is very interesting, but the fact that it is only a small subgroup makes it less valuable. The authors should at least mention why this was and whether anything linked those specimen that could be analysed (as a smaller point in this section please explicitly state that node measurements represent long axis).

We have expanded the discussion regarding the availability of resection specimens for analysis. This can be found in the histopathological methods of the materials and methods section.

We hope you find our responses satisfactory and accept the manuscript for publication.

Kind Regards,

The Authors.

Abstract

Aim

Accurate lymph node staging is vital to inform optimum treatment decisions in patients with oesophageal cancer. This study evaluates the accuracy of contemporary N-staging and provides radiological-pathological correlation in patients with lymph node metastases (LNMs) that were radiologically staged N0.

Materials and Methods

One hundred and twelve patients were included who underwent surgery alone (n=41) or had neo-adjuvant therapy (n=71) between October 2010 and December 2015. Contrast-enhanced CT (CECT), endoscopic ultrasound (EUS) and PET/CT N-stage were compared to pathological N-stage [node-negative (N0) vs node-positive (N+) groups]. Fifty LNMs from 15 patients pre-operatively staged as N0 were measured and the maximum size recorded.

Results

Accuracy, sensitivity and specificity of N0 vs N+ disease with CECT, EUS and PET/CT was 54.5%, 39.7% and 77.3%, 55.4%, 42.6% and 75.0%, and 57.1% 35.3% and 90.9%, respectively. All modalities were more likely to under-stage nodal disease; CECT (X^2 32.890, df 1, $p < 0.001$), EUS (X^2 28.471, df 1, $p < 0.001$) and PET/CT (X^2 50.790, df 1, $p < 0.001$). PET/CT was more likely to under-stage nodal

disease than EUS ($p=0.031$). Median LNM size was 3 mm, with 41 (82%) of LNMs measuring <6 mm and 22 (44%) classified as micro-metastases (≤ 2 mm).

Conclusion

This study has demonstrated poor N-staging accuracy in the modern era of radiological staging. Eighty-two percent of LNMs measured <6 mm, making direct identification extremely challenging on medical imaging. Future research should focus on investigating and developing alternative surrogate markers to predict the likelihood of LNMs.

1 Accuracy of Contemporary Oesophageal Cancer Lymph Node Staging with 2 Radiological-Pathological Correlation

3

4 Introduction

5 Contemporary radiological staging of oesophageal cancer (OC) involves a multi-
6 modality approach. In the UK, patients have initial contrast-enhanced computed
7 tomography (CECT) of the thorax and abdomen following histological confirmation to
8 assess the potential resectability of the tumour, or any distant metastatic disease
9 which may preclude radical therapy.

10 If the patient is deemed suitable for radical treatment, either in the form of definitive
11 chemo-radiotherapy (dCRT) or surgery (+/- neo-adjuvant therapy), positron emission
12 tomography combined with computed tomography (PET/CT) and endoscopic
13 ultrasound (EUS) are performed for a more detailed assessment of disease stage.
14 (1) PET/CT has greater sensitivity for distant metastatic disease than CECT (2),
15 whereas EUS is regarded as the 'gold-standard' investigation for defining T- and N-
16 stage, whilst also assisting surgical and radiotherapy planning. (3)

17 This staging process is complex and time-consuming but necessary, because each
18 modality has limitations for lymph node staging. CECT provides anatomical
19 information only, relies on size criteria and involves radiation. PET/CT also involves
20 radiation but provides additional functional metabolic data and improves the positive
21 predictive value (PPV) of lymph node metastases (LNMs). (4) The differentiation of

22 peri-tumoural LNMs from adjacent avid tumour can be challenging on PET images.
23 (5) This may increase 'false-negative' rates therefore under-staging the extent of
24 nodal disease. EUS has better sensitivity compared to CECT and PET/CT due to its
25 superior contrast resolution.

26 The prognosis of OC is poor, with 5-year survival approximately 13%. (6) Many
27 patients present with advanced disease and the incidence is increasing. (7) The
28 presence of LNMs is a major prognostic indicator, therefore it is vital to stage nodal
29 disease accurately. (8) Accurate staging optimises management plans and provides
30 the best chance of survival for patients with potentially curable disease. If the multi-
31 disciplinary team (MDT) decide upon surgical management and radiological staging
32 is $\geq T3$ or $\geq N1$, two cycles of neo-adjuvant chemotherapy (NACT) are given prior to
33 resection. This is currently considered best practice in the UK, because overall
34 survival was shown to improve compared to surgery alone. (9)

35 Management decisions are influenced by results of lymph node assessment based
36 on findings of radiological staging investigations. Differentiation of node-negative
37 (N0) from node-positive (N+) disease is important, because this should ensure that
38 patients avoid unnecessary chemotherapy if over-staged, and are not denied
39 potentially beneficial neo-adjuvant chemotherapy if under-staged.

40 However, the existence of small LNMs (<6 mm), which cannot be directly visualised
41 on any imaging modality, are likely to cause inaccurate staging and progress, with a
42 subsequent detrimental effect on patient outcome. (10)

43 Therefore, we aim to define the accuracy of CECT, EUS and PET/CT N-stage in the
44 modern era of radiological OC staging. We will also investigate the prevalence of
45 micro-metastases and size of LNMs in patients radiologically staged N0 but
46 pathologically node-positive (pN+), by providing radiological-pathological correlation.

47 **Materials and Methods**

48 This retrospective cohort study includes consecutive patients who underwent
49 surgical resection of an oesophageal or gastro-oesophageal (GOJ) tumour, over a 5-
50 year period (November 2010 – December 2015) within a centralised service.

51 Radiological and pathological staging data was obtained from the *blinded* database
52 (*blinded*) following Regional Upper Gastrointestinal (GI) Cancer MDT discussion.

53 Institutional Review Board (IRB) approval was granted (ref no. 14/WA/1208). The
54 requirement for informed consent was waived.

55 Inclusion criteria were a previously untreated, biopsy-proven oesophageal or GOJ
56 tumour in patients who underwent surgery alone, or had a poor Mandard tumour
57 regression grade (TRG 4) or no response (TRG 5) following either NACT or neo-
58 adjuvant chemo-radiotherapy (NACRT). (11) All patients had fully completed CECT,
59 EUS and PET/CT staging investigations and were classified according to the
60 International Union Against Cancer (UICC) Tumour Node Metastasis (TNM) 7th
61 edition. (12) All patients also had a full pathological N-stage (pN), also defined by
62 the TNM 7th edition.

63 Patients with tumours that showed complete pathological response (pCR, TRG 1) or
64 tumours with some response (TRG 2 & 3) following NACT or NACRT were excluded
65 because the final pathology is not likely to be representative of pre-operative status.
66 Incomplete radiological staging investigations in particular, EUS examinations, in
67 which the operator was unable to traverse a stenotic tumour in order to fully classify
68 N-stage, were excluded. Patients that underwent an 'open-and-close' procedure due
69 to irresectable disease at the time of operation, were also excluded.

70 CECT Acquisition Protocol

71 CECT was performed either in the host institution of the centralised service (*blinded*)
72 or in local referring hospitals prior to surgery, according to Royal College of
73 Radiologists guidelines. (1) All CECT examinations were reviewed at the Regional
74 Upper GI MDT, and deemed to be of a satisfactory technical standard. The
75 technique used at the host institution is as follows: GE HD 750 Discovery 64-slice
76 scanner (GE Healthcare, Pollards Wood, Buckinghamshire, UK); helical acquisition
77 with collimation of 40mm, pitch 0.984:1 and tube rotation speed of 0.4 seconds; tube
78 output of 120kVp with smart mA dose modulation between 60-600mA; slice
79 thickness of 0.625mm; up to 500ml of water orally and 100-150mls of Niopam 300
80 intravenously with bolus tracking. **Lymph nodes were considered involved on CECT**
81 **if the short axis measurement was 1 cm or greater, located in the expected**
82 **distribution of disease, round with loss of fatty hilum and demonstrated altered**
83 **density or enhancement.**

84 EUS Protocol

85 All EUS examinations were performed in 3 centres by 4 endosonographers. At the
86 host institution, an initial endoscopic examination was performed using a 9 mm
87 diameter Olympus Paediatric gastroscope (Olympus, Southend, UK) to assess the
88 degree of oesophageal luminal stenosis. Patients with an estimated oesophageal
89 luminal diameter <15 mm underwent examination using the smaller-diameter MH-
90 908 oesophagoprobe, and where there was no luminal stenosis, the standard UM-
91 2000 echoendoscope was used (Olympus, Southend, UK). The type of
92 echoendoscope used was at the discretion of the endoscopist. The primary

93 oesophageal tumour was assessed, together with an evaluation of peri-oesophageal
94 and peri-gastric structures as described previously. (13) The criteria for malignant
95 lymphadenopathy specified a hypo-echoic pattern, spherical contour, distinct border,
96 and short axis diameter of 6 mm or more.

97 PET/CT Acquisition Protocol

98 Patients were fasted for at least 6 hours prior to tracer administration. Serum glucose
99 levels were routinely checked and confirmed to be less than 7.0 mmol/L prior to
100 proceeding with imaging. Patients received a dose of 4 MBq of ¹⁸F-FDG per kilogram
101 of body weight. Uptake time was 90 minutes, which is standard at our institution. ¹⁸F-
102 FDG PET/CT imaging was performed with a GE 690 PET/CT scanner (GE
103 Healthcare, Pollards Wood, Buckinghamshire, UK). CT images were acquired in a
104 helical acquisition with a pitch of 0.98 and a tube rotation speed of 0.5 seconds.
105 Tube output was 120 kVp with output modulation between 20 and 200 mA. Matrix
106 size for the CT acquisition was 512 x 512 pixels with a 50cm field of view. No oral or
107 intravenous contrast was administered. PET images were acquired at 3 minutes per
108 field of view. The length of the axial field of view was 15.7 cm. Images were
109 reconstructed with the ordered subset expectation maximisation algorithm, with 24
110 subsets and 2 iterations. Matrix size was 256 x 256 pixels, using the VUE Point TM
111 time of flight algorithm. Nodes were classed as involved on PET/CT if identified on
112 the CT component and showed FDG-uptake appreciably higher than background
113 values. No specific standardised uptake value was used for the inclusion of regional
114 nodes. Lymph nodes considered physiological or related to an alternative aetiology
115 were excluded from the N-stage.

116 Histopathological Methods

117 Histopathological reporting of OC specimens was performed according to minimum
118 requirements defined by the Royal College of Pathologists (RCPATH). (14) All lymph
119 nodes identified in the resection specimen were prepared in 3 mm slices for
120 pathological evaluation. N-stage was then assigned depending on the number of
121 LNMs identified. TRG of the primary tumour was assigned according to the degree
122 of fibrosis compared to residual tumour cells. (11) **In discordant cases, all available**
123 **resection specimens that were radiologically staged N0 but pathologically N+ were**
124 **further evaluated. All available specimens were retrieved and reviewed from the**
125 **archive. Due to the retrospective nature of analysis, some of the older cases were**
126 **archived off-site, and were unavailable at the time of evaluation.** The maximum size
127 **(long axis)** of both involved lymph nodes and metastases within those lymph nodes,
128 were retrospectively recorded. Maximum size was defined as the largest dimension
129 on the glass slide measured by a Consultant Pathologist. A micro-metastasis is
130 defined as tumour deposit measuring ≤ 2 mm. (15) Furthermore, a metastasis to
131 lymph node ratio was calculated.

132 Statistical Analysis

133 Descriptive statistics are used to describe categorical and continuous variables. In
134 this study, N-stage is separated into negative (N0) and N+ (N1, N2 or N3) groups.
135 Accuracy is defined as number of correct investigations divided by total number of
136 investigations. Sensitivity and specificity of N+ disease are calculated for each
137 modality. A Chi-square test assessed significant differences in under- or over-
138 staging for each modality. Significant differences in under-staging between

139 modalities was assessed with McNemar's test. A p-value <0.05 was considered
140 statistically significant. Statistical analysis was performed with SPSS v23 (IBM,
141 Chicago, IL).

142 **Results**

143 A total of 190 patients were considered for inclusion in the study. Seventy-eight
144 patients (41.1%) were excluded from the study; 22 were 'open-and-close'
145 procedures, 16 were TRG 1, 13 were TRG 2, 13 were TRG 3 following neo-adjuvant
146 treatment, and 14 had incomplete EUS staging.

147 Following exclusions, 112 patients were included in the study. The median age was
148 65 years (range 24-78) and the male: female ratio was 92 (82.1%): 20 (17.9%).

149 Fifty-nine tumours (52.7%) were located in the oesophagus; 10 in the mid
150 oesophagus and 49 in the distal oesophagus. Fifty-three tumours (47.3%) were
151 located at the GOJ; 19 Siewert (Sw) type I, 15 Sw type II and 19 Sw type III.

152 One hundred tumours (89.3%) were adenocarcinoma, with 11 SCC (9.8%) and 1
153 neuroendocrine (0.9%). Forty-one patients (36.6%) were treated with surgery alone,
154 67 (59.8%) treated with NACT and 4 (3.6%) treated with NACRT. Of the 71 treated
155 with neo-adjuvant therapy, 42 were TRG 4 and 29 were TRG 5.

156 For CECT, 75 patients (67.0%) were staged N0 and 37 (33.0%) were N+. For EUS,
157 72 patients (64.3%) were staged N0 and 40 (35.7%) were N+. For PET/CT, 84
158 (75.1%) were staged N0 and 28 (24.9%) were staged N+. Table 1 compares the
159 frequency of radiological and pathological N-stages for CECT, EUS and PET/CT.

160 Overall, median time between radiological staging and surgery was 3 months (range
161 1-9 months), 1 month (range 0-3 months) in patients undergoing surgery alone and 4
162 months (range 3-4 months) in patients receiving NACT.

163

164 *Accuracy, Sensitivity and Specificity of CECT, EUS and PET/CT N-stage*

165 N0 vs N+ disease was correctly identified with CECT, EUS and PET/CT in 61 cases
166 (54.5%), 62 (55.4%) and 64 (57.1%), respectively. There was no significant
167 difference between CECT, EUS and PET/CT for detecting N+ disease (X^2 0.169, df
168 2, $p=0.919$). The sensitivity and specificity for identifying N0 vs N+ disease with
169 CECT, EUS and PET/CT was 39.7% and 77.3%, 42.6% and 75.0%, and 35.3% and
170 90.9%, respectively.

171 Under-staging vs Over-staging

172 All modalities were significantly more likely to under-stage nodal disease; CECT (X^2
173 32.890, df 1, $p<0.001$), EUS (X^2 28.471, df 1, $p<0.001$) and PET/CT (X^2 50.790, df 1,
174 $p<0.001$). Comparing modalities, there was a borderline significant difference in
175 under-staging between CECT and EUS ($p=0.063$) but no difference between CECT
176 and PET/CT ($p=1.000$). However, there was a statistically significant between both
177 EUS with PET/CT ($p=0.031$), suggesting PET/CT may further under-stage nodal
178 disease.

179 Pathological Lymph Node Measurement

180 Fifteen archived resection specimens in patients pre-operatively staged N0 were
181 available for retrospective measurement of the lymph nodes and their respective
182 metastases. In total, 50 involved lymph nodes were assessed. (Table 2) The median

183 size of involved lymph nodes was 6 mm (range 2-15 mm) and the median metastasis
184 size was 3 mm (0.5-13.5 mm). Twenty-two (44%) LNMs measured ≤ 2 mm, which
185 are defined as micro-metastases. (Fig. 1) Forty-one (82%) LNMs were ≤ 6 mm and
186 46 (92%) LNMs were ≤ 10 mm. A metastasis to lymph node size ratio was
187 calculated. Thirty-one (62%) of the lymph nodes examined were replaced with $\geq 50\%$
188 metastatic deposit, 19 (38%) were replaced with $< 50\%$ metastatic deposit, with 12
189 (24%) replaced with $< 25\%$ metastatic deposit, using maximum size criteria.

190 **Discussion**

191 This study has found poor N-stage accuracy with CECT, EUS and PET/CT. In
192 general, all modalities were more likely to under-stage nodal disease, with PET/CT
193 more likely to under-stage than EUS. Another important finding, is the prevalence of
194 small LNMs (<6 mm) in the resection specimens of patients radiologically staged N0.
195 Micro-metastases have been found in lymph nodes of early oesophageal tumours
196 (16) but little has been published with radiological correlation. Studies investigating
197 lung cancer have detected micro-metastases in patients radiologically staged N0
198 (17), although evidence in OC is lacking.

199 The majority of LNMs (82%) were <6 mm, which makes direct visualisation
200 extremely challenging on current medical imaging techniques and is likely to be the
201 main reason for discrepancy between radiological and pathological staging. In
202 addition, traditional radiological measurement of lymph nodes is taken in the short-
203 axis (18), which further reduces the likelihood that LNMs are diagnosed. Even with
204 the improved contrast resolution of EUS compared to cross-sectional imaging, it is
205 unlikely that a lymph node of this size would confidently be classified as involved.
206 (13) Similarly, there was a relatively high prevalence of micro-metastases (44%).

207 These results have significant implications for treatment decision-making processes
208 and demonstrate that contemporary radiology techniques are inadequate for N-
209 staging. Numerous studies have demonstrated the importance of LNMs, which have
210 a significant effect on overall survival. (8) Better evidence is required to understand
211 the prognostic significance of micro-metastases, but they are generally felt to confer
212 a worse prognosis. (19, 20)

213 There is evidence that a significant proportion of surgical patients have systemic
214 micro-metastases at the time of resection. In one study, micro-metastases were
215 detected in the resected rib in 53.7% to 78% of cases, and was dependent on the
216 histological technique used. (21) This is a higher detection rate than our study, but
217 the results are comparable due to different techniques and tissues used between the
218 studies. The high rate of micro-metastases may be a reason that our results show
219 significant under-staging of nodal disease, and perhaps clinicians could consider
220 lowering the threshold for treating patients with systemic neo-adjuvant therapy.

221 Previously published research from our institution has shown the prognostic
222 significance of N-stage, LNM count and volume of nodal disease in patients with OC.
223 (22, 23) Nodal disease in these studies probably continues to be an important
224 prognostic indicator, but the radiological staging is likely to have under-estimated the
225 total nodal disease burden in those patient cohorts. Results of staging performance
226 have also been published from our institution. These studies compared CECT and
227 EUS with pN-staging. Blackshaw et al focused on accuracy of N-staging in GOJ
228 tumours and found significant differences in agreement, sensitivity and specificity
229 between Siewert type II and type III tumours. (24) Weaver et al found agreement,
230 sensitivity and specificity of N-staging was 0.603, 79% and 84% for CECT and
231 0.610, 91% and 68% for EUS. (13) The results of the current study show poorer
232 agreement and sensitivity. There are a number of reasons for these findings,
233 including disease evolution, greater inter-observer variability between reporters, and
234 fewer, but more specialised upper GI cancer pathologists reporting the resection
235 specimens, with possibly higher rates of LNM detection. (15)

236 Accuracy of diagnosing N+ disease with CECT, EUS and PET/CT was 54.5%,
237 55.4% and 57.1%, respectively. In a clinical context, these results are unsatisfactory
238 given that the presence of LNMs is such a major prognostic indicator. (8) The
239 sensitivity and specificity for identifying N0 vs N+ disease with CECT, EUS and
240 PET/CT was 39.7% and 77.3%, 42.6% and 75.0%, and 35.3% and 90.9%.
241 Specificity results are comparable with past meta-analyses but sensitivity results are
242 lower for all modalities. Previously published literature states sensitivity for N-
243 staging of EUS, CECT and PET/CT is 80%, 50 % and 57%, and specificity is 70%,
244 83% and 85%, respectively. (2) However, this meta-analysis was conducted prior to
245 this centralisation of many upper GI cancer services. The reduced sensitivity of
246 staging investigations is supported by our results, which demonstrate that under-
247 staging is more common for all modalities.

248 As current investigations are unreliable for differentiating N0 from N+ disease, future
249 research should focus on investigating and developing new methods of predicting
250 the likelihood of lymph node involvement. Surrogate markers of LNMs, such as
251 texture analysis of the primary tumour and other non-invasive quantitative imaging
252 techniques, may allow better risk stratification of patients, provide more powerful
253 prognostic data and further inform optimum treatment decisions. (25, 26) MRI may
254 provide an alternative staging modality. Research studies have demonstrated
255 variable diagnostic ability, with sensitivity, specificity and accuracy ranging between
256 38-62%, 68-85% and 64-77%, respectively. These current results are comparable to
257 CT, EUS and PET/CT but continuing improvements in functional MRI scanner
258 technology may yield further developments. (27, 28)

259 Strengths of Study

260 This study provides radiological-pathological correlation in a group of OC patients
261 with discordant nodal staging. Radiological-pathological correlation is essential for
262 understanding limitations of staging techniques and identifies areas requiring further
263 research. All patients were discussed at the Regional MDT and the management
264 plan for each individual was decided upon by consensus. The Regional MDT covers
265 a large population of over 1.4 million people and is highly experienced in the
266 management of OC. Histopathological examination was performed by consultant GI
267 pathologists according the guidelines defined by the RCPATH. (14) We implemented
268 strict criteria to control the selection of patients for this study, which compares
269 imaging findings to 'gold-standard' pathological staging. The majority of patients
270 received neo-adjuvant therapy, which can alter the stage of disease between pre-
271 treatment imaging and surgical resection. To control for this, only patients with
272 Mandard TRG 4 or 5 were included, which should allow a more direct comparison
273 with the final pathological resection specimen. The majority of patients tend to have
274 a TRG 4 or 5 response. (29)

275 Limitations

276 As a result of neo-adjuvant therapy, there is a time-lag between radiological staging
277 and surgical resection, which could allow for tumour progression and LNM
278 development. However, the median time period in this study was 3 months. In
279 addition, patients with an 'open-and-close' procedure were excluded, which further
280 demonstrates radiological disease under-staging. There are also known limitations
281 of pathological lymph node examination. Approximately 3 mm sections are taken
282 through lymph nodes once they are mounted in a cassette, but this may be
283 performed with varying skill and consistency. Micro-metastases may be missed if

284 not bisected during preparation, and this suggests that the true incidence of micro-
285 metastases in this cohort of patients may be even greater. Although the RCPATH
286 define the minimum requirements for pathological reporting, there is no
287 recommended, standardised method for lymph node preparation and assessment in
288 OC, at present. The centralised upper GI cancer service is referred patients from
289 several local NHS trusts. As a result, multiple readers from different hospitals report
290 the staging CECT examinations. During this period, 3 endosonographers performed
291 the EUS examinations in 2 different hospitals. All PET/CT scans were performed
292 using the same scanner and protocol and were reported by 4 different consultant
293 radiologists. However, all staging was performed according to the TNM 7th edition.

294 **Conclusion**

295 In conclusion, this evaluation of contemporary staging performance over a 5-year
296 period in a centralised upper GI cancer service has shown poor N-staging accuracy
297 for CECT, EUS and PET/CT. Radiological-pathological correlation in patients staged
298 N0 has shown a large number of small LNMs (<6 mm) that are extremely
299 challenging to diagnose directly from medical imaging. The findings of this study
300 have significant implications for patient care, because radiological staging results
301 largely influence treatment decisions made by the MDT. Future research should
302 focus on prediction of the likelihood of lymph node involvement as current lymph
303 node imaging is inadequate.

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395

396 **Figure Legends**

397 **Figure 1.** CT (with calipers), PET and fused PET/CT images of a 'false-negative' left
398 gastric lymph node in a patient with junctional adenocarcinoma. A low-power
399 magnification of the lymph node shows a micro-metastasis. For reference, the
400 lymph node measured 5 mm in maximum size and the micro-metastasis (highlighted
401 with yellow outline) measured 1.2 mm.

402 **Table 1**

403 Table 1. Comparison of N-stage frequency classified by CECT, EUS, PET/CT and
 404 pathology.

CECT N-stage					
Frequency (%)	N0	N1	N2	N3	Total
pN0	34 (30.4)	8 (7.1)	2 (1.7)	0 (0.0)	44 (39.3)
pN1	21 (18.8)	4 (3.6)	2 (1.7)	0 (0.0)	27 (24.1)
pN2	16 (14.3)	10 (8.9)	1 (0.9)	0 (0.0)	27 (24.1)
pN3	4 (3.6)	7 (6.3)	3 (2.7)	0 (0.0)	14 (12.5)
Total	75 (67.0)	29 (25.9)	8 (7.1)	0 (0.0)	112 (100.0)

EUS N-Stage					
Frequency (%)	N0	N1	N2	N3	Total
pN0	33 (29.5)	9 (8.0)	1 (0.9)	1 (0.9)	44 (39.3)
pN1	20 (17.9)	7 (6.3)	0 (0.0)	0 (0.0)	27 (24.1)
pN2	13 (11.6)	10 (8.9)	4 (3.6)	0 (0.0)	27 (24.1)
pN3	6 (5.4)	6 (5.4)	1 (0.9)	1 (0.9)	14 (12.5)
Total	72 (64.3)	32 (28.6)	6 (5.4)	2 (1.7)	112 (100.0)

PET/CT N-stage					
Frequency (%)	N0	N1	N2	N3	Total
pN0	40 (35.8)	4 (3.6)	0 (0.0)	0 (0.0)	44 (39.4)
pN1	23 (20.5)	4 (3.6)	0 (0.0)	0 (0.0)	27 (24.1)
pN2	15 (13.4)	10 (8.9)	2 (1.7)	0 (0.0)	27 (24.1)
pN3	6 (5.4)	6 (5.4)	2 (1.7)	0 (0.0)	14 (12.5)
Total	84 (75.1)	24 (21.4)	4 (3.6)	0 (0.0)	112 (100.0)

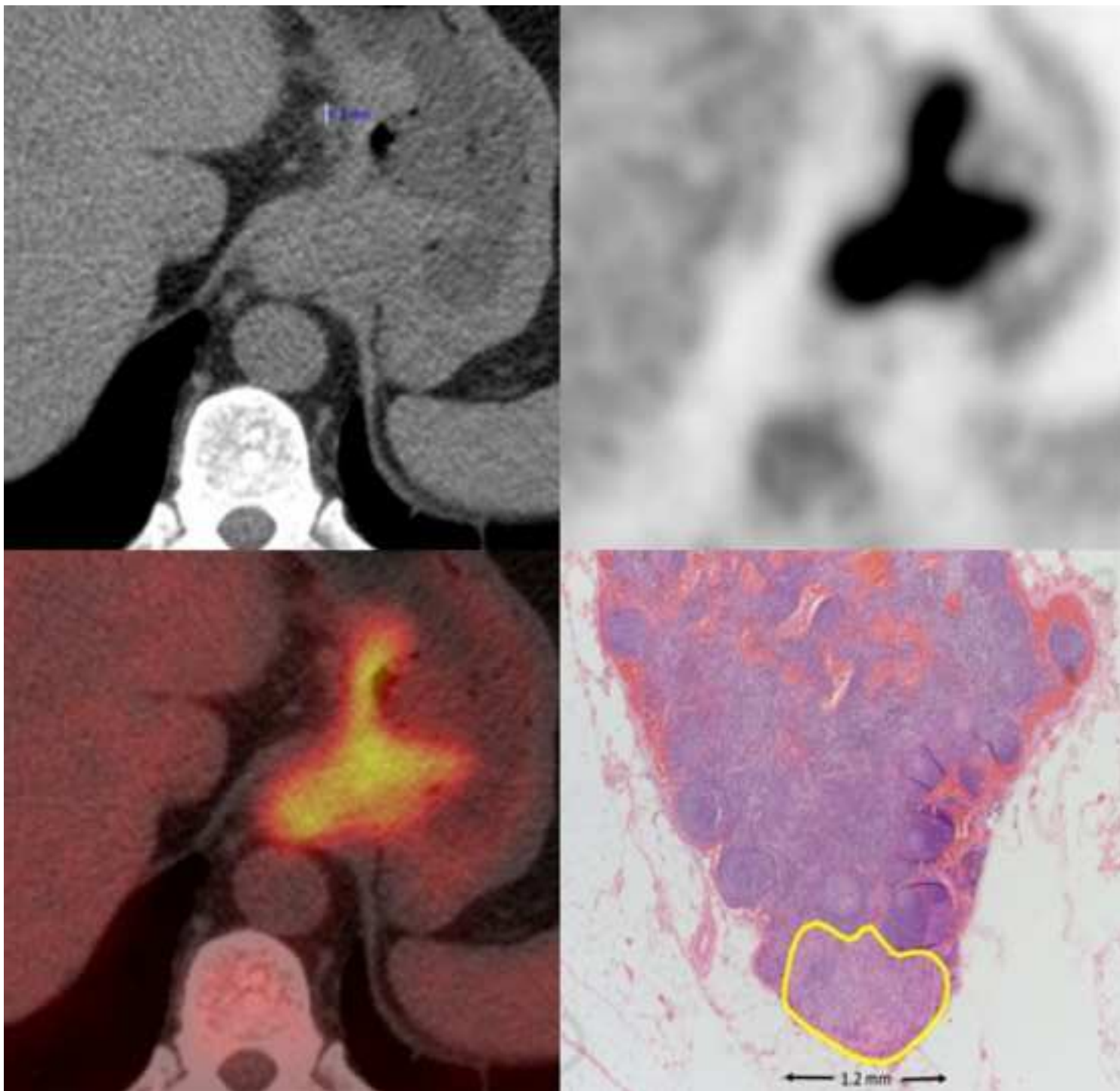
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406 **Table 2**

407 Table 2. Frequency of and distribution of lymph node and metastasis size when
 408 separated in groups of 2 mm for descriptive purposes.

Frequency (%)	Maximum Size (mm)							
	0-2	2.1-4	4.1-6	6.1-8	8.1-10	10.1-12	12.1-14	14.1-16
Lymph Node	3 (6.0)	11 (22.0)	13 (26.0)	12 (24.0)	4 (8.0)	3 (6.0)	3 (6.0)	1 (2.0)
Metastasis	22 (44.0)	9 (18.0)	10 (20.0)	3 (6.0)	2 (4.0)	2 (4.0)	2 (4.0)	0 (0.0)

409



Highlights

1. CT, EUS and PET/CT N-staging accuracy is poor in oesophageal cancer.
2. CT, EUS and PET/CT are all more likely to under-stage nodal disease.
3. Many lymph node metastases are too small to be identified with direct imaging.