REVIEWER RESPONSE:

Name of Journal: *World Journal of Gastrointestinal Endoscopy*

ESPS Manuscript NO: 33116

Manuscript Type: Review

Endoscopic Ultrasound in Oncology: an update of clinical applications in the gastrointestinal tract

Valero M *et al. Endoscopic Ultrasound in Oncology*

Manuel Valero, Carlos Robles-Medranda

REVIEWER RESPONSE:

Dear Editor and Reviewers: thanks again for the opportunity and the comments made to our paper entitled: "Endoscopic Ultrasound in Oncology: an update of clinical applications in the gastrointestinal tract".

The paper was revised and the modifications suggested were done. Below you can find all the modifications step by step with the comments:

Reviewer 03259215 comments:

Response:

Totally agree with your comments. In this revision and resubmission you will find a paragraph with the mention of the American College Guideline (reference 32). In addition, more explanation of the figures were done as you can see below and in the final edited version

Reviewer 03026750 comments:

Response:
Thanks for your comments. We added two more figures in this resubmission and revision one of eus elastography and one with eus-contrast enhancement in accordance to your suggestions, as you can see below and in the final edited version

Response to the editor:
The references were corrected in accordance to your suggestions and comments. Finally the audio core tip and the signed conflict of interest pdf was attached.

Best Regards,

Carlos Robles-Medranda, MD

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Endoscopic Ultrasound in Oncology: an update of clinical applications in the gastrointestinal tract

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Conflict-of-interest statement: The authors have no conflict of interests.

批注 [W用1]: Please offer signed pdf file. Thank you!

批注 [CR2]: A pdf file signed was uploaded.

ESOPHAGEAL CANCER

Characteristics of esophageal cancer and clinical implications

The prognosis of esophageal cancer (EC) is poor because these tumors are usually detected in an advanced stage. Surgery is not possible in most cases and has a high rate of morbidity and mortality. The level of tumor invasion and lymph node metastasis will determine treatment and prognosis. Therefore, EUS plays a vital role by providing an accurate T and N staging, which allows deciding on the best treatment [7]. The use of EUS evaluation in preoperative staging has led to a mortality reduction of 42.1% and a better recurrence-free survival rate, compared to patients with no EUS evaluation [8]. According to the TNM classification **(Table 1)**, superficial esophageal cancer includes mucosal and submucosal involvement (Tis, T1a or T1b) [9]. Patients with any nodal involvement (N+) or advance tumors (T2 – T4a) **(Figure 1)** need preoperative neoadyuvant chemoradiotherapy, whereas T1 patients with no nodal metastasis can benefit from endoscopic (Tis, T1a N0) or surgical resection (T1bN0) [10-12]. When different staging methods were compared, CT, MRI and PET-scan showed themselves to be better than EUS in evaluating distant metastasis (M), however EUS proved superiority in the detection of tumor stage

批注 [CR3]: Figure 1

(T) and lymph nodes (N) [13-16]. One method does not have to exclude the other. The incorporation of CT, PET and EUS in preoperative staging reduces the number of unnecessary surgical procedures from 44% to 21 % [17].

The role of EUS in T staging

EC limited to the mucosa (Tis, T1a) can be treated effectively with minimally invasive endoscopic therapy, whereas submucosal (T1b) EC carries relatively high risk of lymph node metastasis and requires surgical resection. According to a meta-analysis by Puli et al. (49 articles), EUS sensitivity and specificity for T stage was 81.6% and 99.4%, for T1, 81.4% and 96.3%, for T2, 91.4% and 94.4%, for T3, and 92.4% and 97.4% for T4 staging, respectively. The accuracy was higher for T3 - T4 lesions (>90%) than T1 – T2 (65%) [18]. However, a study by Thosani et al. reported, on the analysis of 1019 patients with only superficial EC, that EUS sensitivity and specificity was 85% and 87% for T1a and 86% and 86% for T1b respectively, with an overall EUS accuracy for superficial EC staging of >93% [19].

The role of EUS in N staging

The lymph node (LN) metastasis in EC is considered the main fact that influences prognosis and it depends on the number of nodes involved. This pathology has a high rate of lymph node (LN) involvement at an early stage. T1sm (T1b) disease has a 15% to 30% rate of LN dissemination. The 7th edition of the AJCC **(Table 1)** classifies the N stage according to the number of metastasized lymph nodes in N1 (1 to 2), N2 (3 to 6), and N3 (≥7). The use of EUS evaluation in preoperative staging has led to a mortality reduction of 42.1% and a better recurrence-free survival rate, compared to patients with no EUS evaluation [8]. According to the TNM classification **(Table 1)** [9], the presence of node metastasis indicates the need of neoadjuvant therapy. Therefore, identification of the N stage is mandatory. PET and CT have a low accuracy (51%) compared to EUS [20]. The evaluation of the LN features using EUS have shown that malignant nodes tend to be larger than 1 cm, round, sharply

demarcated, and hypoechoic. When all these features are present there is an 85% chance of malignancy. However, only 25% of malignant LN have all four features [21]. A systematic review found that EUS has a sensitivity range of 59.5% to 100% and a specificity range of 40% to 100% for N staging [22]. Puli et al. described a EUS sensitivity for N stage of 85% and also showed that the use of Fine Needle Aspiration (FNA) substantially improves the sensitivity and specificity of EUS nodal staging from 85% to 97% and 85% to 96% respectively, with a low rate of complications, ranging from 0% to 2.3% [18]. Chen et al. found an accuracy rate of 99.4% using EUS-FNA [23]. In patients with EC, the identification of a celiac lymph node is synonymous to LN metastasis in 90% of the cases regardless of echo features and size and therefore indicates a poor prognosis [24]. EUS-FNA for celiac lymph node diagnosis has shown a sensitivity of 72% to 83%, a specificity of 85% to 98%, and an accuracy of 94% [25].

Limitations

The role of EUS has some limitations. It may be less accurate for assessing the T1 - T2 stage compared with T3 - T4. According to some authors there is a trend to overstaging the depth of the submucosal invasion, with a low accuracy rate in early T staging (64%) [26]. The use of high frequency catheter probes may improve the diagnostic accuracy in early lesions from 83% to 92%, but the results are heterogeneous [27,28]. EUS criteria are not accurate after neoadjuvant radio-chemotherapy because EUS poorly differentiates tumor from necrosis or inflammatory reaction [29]. The presence of esophageal malignant stenosis that cannot be overcome can make TNM evaluation more difficult. A recent multi-center study suggested that routine EUS examinations may not be required in all patients with EC as the inability to advance a diagnostic gastroscope through a malignant stricture correlates 100% with locally advanced disease, so that performing a EUS does not change the treatment decision [30].

Role of EUS in Barrett's esophagus

EUS has long been used to evaluate Barrett´s esophagus (BE) [6]. In the case of

BE associated with high-grade dysplasia (HGD) or early (T1m) esophageal adenocarcinoma (EAC), the patient may benefit from endoscopy resection, but if EUS shows an advanced disease with tumor invading the submucosal, or beyond, or lymph node involvement, endoscopic therapy may not be warranted. Qumseya et al. showed in a recent meta-analysis that 14% of patients referred to EUS for BE associated with HGD or EAC will have advanced cancer (>T1sm or >N1) detected by EUS that is not amenable to endoscopic treatment and which therefore changes the therapeutic approach. With EUS it was found that 4% of these patients have advanced disease in the absence of nodules. The sensitivity and specificity for T stage was 56% and 89% and for N stage was 71% and 94 % respectively [31]. However, even the data mentioned, the American College of Gastroenterology has stated that EUS routine staging of patients with BE before EMR is unwarranted as clinical decision making will rest with the EMR findings and given the possibility of over- and understaging in patients with superficial EAC [32-35]. In case of T1a lesions the rate of lymph node (LN) involvement is low, making these lesions optimally treated by EMR [36,37]. In patients with known T1b sm1 disease, there is conflicting data with respect to the likelihood of LN invasion [38,39]. The evidence of LN involvement, especially if substantiated by FNA, means that any attempt at endoscopic therapy would be palliative and therefore EUS may have a role in assessing and sampling regional LN, given the increased prevalence of lymph node involvement in these patients compared with less advanced disease [19].

批注 [CR4]: Reference 32 was added in accordance to reviewer 33116 comments

批注 [CR5]: This paragraph regarding the American College guideline was added, in accordance to the suggestions of reviewer 33116. We also added the reference

GASTRIC CANCER

Characteristics of gastric cancer and clinical implications

Gastric cancer (GC) is the fourth most common cancer and the second cause of cancer-related deaths (10%) [40]. An accurate staging **(Table 2)** can be extremely useful in providing patients with the best therapeutic option. Patients with

early gastric cancer (EGC), in the presence of favorable prognosis features (well-differentiated carcinoma, limited to the mucosa, diameter <2 cm, absence of ulceration) and no lymph node involvement (N0) can benefit from endoscopic resection rather than surgical resection [41, 42]. On the other hand, patients with advanced gastric cancer (AGC) (T3 - T4 tumors or N+) need to be treated with neoadjuvant therapy (chemotherapy, radiotherapy or both) [43, 44].

Computed tomography (CT) is a frequent imaging method for the preoperative staging of GC [45]. It has a high accuracy for distant metastasis (M), however its overall accuracy for loco-regional staging (T and N stages) is low, ranging from 65% to 85% [46, 47]. The CT sensitivity and specificity for N stage is 77% and 78%, respectively [48]. No better results appear to be achievable with magnetic resonance imaging (MRI) or positron emission tomography (PET) [48-50].

Thus these imaging devices are mostly used to diagnose locally advanced lesions (T3 - T4 or N+) or distant metastasis than early stages of GC. On the contrary, EUS is an accurate device for the loco-regional staging [51,52]. **(Figure 2)**

批注 **[CR6]:** Figure 2

The employment of EUS in the preoperative stage of GC has shown to change the therapeutic management in 30% of cases, resulting in more limited surgical resections, especially in stages T1 and T3 [53].

The role of EUS in T staging

A recent meta-analysis by Mocellin and the Cochrane Collaboration Group (2015) evaluated 66 articles (n: 7747) about gastric cancer staged with EUS. The aim was to evaluate EUS ability to separate patients with GC who would best benefit from surgery without preoperative radio-chemotherapy (T1 - T2) from those with advanced tumors (T3 - T4) who are likely to benefit from neoadjuvant therapy. They found EUS sensitivity and specificity to discriminate T1 - T2 from T3 - T4 lesions to be 86% and 90% respectively. A second analysis was made in order to evaluate EUS ability to discriminate between patients with superficial cancers (T1 from T2 and T1a from T1b), with the intention of identifying patients who would benefit from endoscopic resection rather than surgery. The sensitivity and specificity of EUS to distinguish T1 (early gastric

cancer) from T2 (muscle-infiltrating) was 85% and 90% respectively. As for the capacity of EUS to distinguish between T1a (mucosal) versus T1b (submucosal), they showed that the sensitivity and specificity was 87% and 75% respectively. They concluded that EUS can distinguish between superficial (T1 - T2) and advanced (T3 - T4) primary tumors with a sensitivity and specificity greater than 85%. This performance is maintained for the discrimination between T1 and T2 superficial tumors. However, EUS diagnostic accuracy is lower when it comes to distinguishing between the different types of early tumors (T1a vs. T1b) [54]. This conclusion correlates with Mocellin et al. previous results (2011) when they described that EUS can differentiate T1-2 from T3-4 GC with high accuracy (sensitivity of 86% and specificity of 91%) [55]. Cardoso et al. (2012) also showed that EUS seems to identify advanced T stage (T3 and T4) better than it identifies less advanced T stage or N stage, with a combined accuracy for T staging of 75% [56]. Puli et al. (2008) evaluated 22 studies (n: 1896) and described the usefulness of EUS in GC. The sensitivity and specificity by stage were, 88.1% and 100% for T1, 82.3% and 95.6% for T2, 89.7% and 94.7% for T3, and 99.2% and 96.7% for T4. Incidentally, EUS for T stage detection was more accurate in advanced cancer than in early cancer [57]. Kwee et al. (2008) showed in a systematic review (18 studies), the accuracy of EUS in differentiating mucosal (T1m) from deeper GC (>T1sm) and found that sensitivity and specificity of EUS in detecting cancerous extension beyond the mucosa ranged from 18.2 to 100% (median 87.8%) and from 34.7 to 100 % (median 80.2%) respectively. They concluded that the studies showed too much heterogeneity and it is still unclear whether EUS can accurately differentiate between mucosal and deeper gastric cancer [58].

The role of EUS in N staging

The accuracy of EUS for N staging has shown remarkable heterogeneity of results. Mocellin et al. described after the evaluation of 44 studies (n = 3573) an overall sensitivity and specificity of 83% and 67% respectively [54]. Cardoso et al. reported accuracy for N stage of 64%, sensitivity of 74%, and specificity of 80%.

These results were due to the low possibility of detecting metastasized lymph nodes that are distant from the lesion [56]. Kwee et al. found that sensitivity and specificity of EUS varied from 16.7% to 95.3% (median 70.8%) and 48.4% to 100% (median, 84.6%) [59]. Puli et al. after the analysis of 22 studies (n=1896) reported a sensitivity for N1 of 58.2% and N2 of 64.9%. The pooled sensitivity to diagnose distant metastasis was 73.2% [57].

Limitations

There is a remarkable heterogeneity of the evidence currently available about the ability of EUS to differentiate T1a vs. T1b tumors and to diagnose lymph node metastasis (N0 versus N+). Therefore, physicians should be cautious at the time of interpreting these results. Tumor features like size and location may affect diagnostic performance of EUS. A tumor size greater than 3 cm is associated with overstaging by EUS and decreases the diagnostic accuracy to 50% [60]. The cardia, the greater curve of upper body, the lesser curve at the incisura and the pyloric channel are the most challenging areas to examine [61].

Gastric Lymphoma

Even though CT has proved useful for evaluating an abnormal gastric wall thickening, EUS, on the other hand, has shown itself to be superior for examining nodal involvement, extension and depth of tumor invasion [62]. The EUS diagnostic accuracy in gastric lymphoma is 91- 95% for T stage and 77 - 83% for N stage [63, 64]. The use of EUS-FNA combined with flow cytometry and immunohistochemistry can improve N staging accuracy substantially [65].

EUS has also shown a significant impact on treatment decisions. Gastric lymphoma confined to the mucosal and submucosal (T1) can simply be treated with H. Pylori eradication therapy. However, if EUS shows deeper invasion, chemotherapy, radiation or surgical treatment may be necessary [66]. Moreover, EUS has proven to be useful for surveillance of recurrences at an early stage [62].

批注 **[CR7]:** Figure 2 in this paragraph was removed because there was a mistake. Our figure 2 was an adenocarcinoma and not a lymphoma

RECTAL, COLON AND ANAL CANCER

Characteristics of rectal cancer and clinical implications

Accurate staging in Rectal Cancer (RC) is crucial for choosing the best multimodal therapy. Treatment decisions and prognosis depends on both T and N stage of the disease at the time of diagnosis [67]. In the absence of distant metastasis (M), EUS is the most accurate imaging modality for loco-regional staging (T and N stages) of rectal tumors [68]. Stage I disease includes early rectal lesions (T1-T2 N0 M0). **(Table 3)** While T1 lesions can benefit from endoscopic mucosal resection or transanal endoscopic microsurgery, T2 lesions need surgery [69, 70]. Stage II disease with locally advanced cancer (T3-T4 N0 M0), or stage III with lymph node metastasis (T1-4 N1-2 M0) will benefit maximally and improve recurrence-free survival when neoadjuvant radio-chemotherapy is given [71-74]. Preoperative biopsies of rectal tumors may fail to diagnose an invasive carcinoma, with up to 24% false negative results. The preoperative use of EUS reduces the rate of missed carcinomas from 21% to 3% [75]. EUS compared to other imaging modalities (CT, PET/CT, MRI) is superior and more accurate in determining T stage (EUS: 87%, CT: 76% and MRI: 77%) [70,76-77]. In N stage situations, it is also superior, but the difference is less obvious and accuracy varies between studies (EUS 63%-85%, CT 56%–79% and MRI 57%–85%) [78-82]. Usually CT and PET/CT are used for distant metastasis diagnosis [82]. It is also reported that when CT was the original mode of investigation but a further EUS was done, in 31% of the cases the mode of treatment was changed because of the result [70]. The combination of CT and EUS seems to be the most cost-effective diagnostic strategy [83]. MRI has less accuracy in the T stage than EUS does, but provides a good definition of the circumferential resection margin (CRM). While EUS is more useful for staging early RC, MRI is indicated for staging advanced disease and defines CRM. Also, it can be used in the case of stenotic tumors, when EUS is less accurate. Thus, EUS and MRI are complementary and should be both used for preoperative staging [81, 84].

RC recurrence rates range from 20% to 50%, depending on how advanced the cancer is and if neoadjuvant therapy has been administered before surgery [85,86]. It has been proven that there is a significant reduction in tumor recurrence when patients undergo EUS staging compared to those who do not [87]. In addition to this, EUS can be used to evaluate the colorectal anastomosis during follow-up of patients operated for RC and confirm or rule out recurrence with 97% sensitivity, 100% specificity, 100% positive predictive value (PPV), 94% negative predictive value (NPV), and an overall accuracy of 98% [88, 89]. One limitation that has been attributed to EUS is its difficulty in differentiating between post-operative benign lesions and recurring cancer in postoperative lesions. However, the use of EUS-guided FNA increases the specificity from 57% to 97% [85, 86]. Thus, EUS has a main role in both preoperative staging and follow-up after surgery.

The role of EUS in T staging

Over- or under-staging leads to changes in a patient´s treatment. Surgery instead of endoscopic resection and the use of chemoradiotherapy could be wrongly indicated when there is over-staging. On the other hand, under-staging with the lack of neoadjuvant indication could lead to an insufficient treatment. According to a recent review performed by Marone et al. (33 articles, n: 4976), EUS assesses the tumor penetration depth into the rectal wall with an overall accuracy for T stage of about 84%, ranging from 63% to 96%, while the reported accuracy of CT and MRI are 65%-75% and 75%-85%, respectively. They showed also that EUS accuracy for T stage is strictly related to the depth of infiltration, being lower for T2 stage than for early (T1) or advanced (T3-4) RC (T1: 88%, T2: 78.4%, T3: 85.4% and T4: 80.2%) [90]. Similarly, a meta-analysis (42 studies, n: 5039 patients) showed that EUS has an overall RC staging sensitivity of 81%-96% and specificity of 91%-98%, showing higher sensitivity for advanced RC (95%) than early cancer (88%). The pooled sensitivity and specificity by stage was for T1: 88% and 98%, T2: 81% and 96%, T3: 96% and 91% and T4: 95% and 98%, respectively. The authors concluded that EUS should be

the imaging method of choice for the T staging of RC [91]. **(Figure 3)** Superficial RC limited to the mucosa can be resected endoscopically. EUS has a high accuracy rate in differentiating T1 from T2 lesions, ranging from 81% to 95%, with an overstaging or understaging rate of 9% [92]. Puli et al. evaluated, in a meta-analysis (11 studies, n: 1791), the efficacy of preoperative EUS in staging patients with RC confined to the mucosa (T0) and found that sensitivity was 97% and specificity 96%. They concluded that EUS should be strongly considered for staging of early rectal cancers [93].

批注 **[CR8]:** Figure 3

The role of EUS in N staging

EUS role in the determination of lymph node (LN) metastasis is less precise than T staging, with a mean accuracy of 74% (range 63%-85%) [90]. However, the accuracy is still better than others imaging modalities like CT (56–79%) or MRI (57–85%) [78-82]. Similarly, a meta-analysis including 35 articles showed that EUS has a sensitivity of 73% and specificity of 76% for N staging. This low EUS performance is related to the difficulty in evaluating distant metastatic LN that are out of EUS scanning, discriminating between inflammatory and metastatic LN and the tendency to overlook small metastatic LN compared to larger LN [94-98]. The presence of all malignant features (enlarged node ≥1 cm, hypoechoic appearance, round shape, and smooth border) is related to 100% of PPV for malignancy, however this situation is seen in less than 25% of cases [21]. It is known that there is a correlation between T stage and risk of LN involvement in patients with RC. The risk varies from 6%-11% for T1, 10%-35% for T2 and 26%-65% for T3-T4 RC [99]. Similarly, the EUS accuracy for N staging also depends on T staging and seems to be better for advanced disease (84% in T3 compared to 48% in T1). This is explained by the fact that in T1 lesions metastatic nodes are possibly small [98]. On the other hand, beside EUS limitations in N staging, EUS guided FNA can be used to balance and improve the accuracy from 75% to 87% [100]. EUS-FNA has a sensitivity, specificity, PPV and NPV of 89%, 79%, 89% and 79% respectively [97, 101]. The fact that EUS-FNA has a moderate NPV (77%) for N staging means that LN metastases cannot be ruled out by a negative FNA [102].

Despite the fact that most perirectal nodes detected by EUS in patients with RC are metastatic, it is important to confirm this. EUS-FNA should be indicated when results change the therapeutic strategy. The presence or absence of LN metastasis in T1-T2 lesions change the stage of the patient from I to III and indicates the chemoradiotherapy strategy. EUS-FNA changes patient management in 19% of the cases [70, 103].

Limitations

EUS performance is operator-dependent and accuracy improves with experience. This fact explains the wide range of overall accuracy for T and N staging between studies (63% to 95%) [104, 105]. A high inter-observer variability (61%-77%) has been described according to the experience of the operator, with overstaging values of 19% and understaging of 12% [104]. Also, EUS seems to be less accurate in restaging RC after neoadjuvant therapy (NAT), due to the limitations in differentiating inflammation, edema, necrosis and fibrosis from neoplastic infiltration, with the risk of overstaging and overtreatment [68,106,107]. EUS correctly predicts complete response to chemoradiation in 50-63% of the cases. It has an overall accuracy for T stage of 48%, with 38% of overstaging and 14% of understaging [108, 109]. Another limitation is that in 14% of RC there is a stricture that cannot be traversed by the echoendoscope, leading to an inaccurate T and N staging. The presence of a stricture decreases the EUS accuracy rate for T stage from 93% to 56%. When the T stages were analyzed separately, the accuracy was 76% for T1, 72% for T2, 91% for T3 and 67% for T4 stage. Moreover, there was an 11% of over-staging and 5% of under-staging errors [110]. Ultrasound catheter probes can be used to compensate this limitation. A meta-analysis (10 studies, n: 642) showed a high performance using ultrasound catheter probes for T and N staging. The pooled sensitivity and specificity were for T1: 91% and 98%, T2: 78% and 94%, T3-T4: 97% and 90%, respectively. The sensitivity and specificity for N staging were 63% and 82%, respectively [111]. Finally, the circumferential resection margin (CRM) is an important factor in predicting local recurrence. MRI has been described to have a better overall accuracy compared to EUS (92% vs. 84%) with similar NPV

(97%), especially in mid-rectum [112]. However, in low RC the accuracy in both modalities is similar (87%) with a NPV of 96% [113].

New Technologies

EUS elastography is a software application that can analyze the elastic properties of tissues. **(Figure 4)** Harder tissue (usually malignant) appears blue which allows one to distinguish between adenocarcinomas and adenomas with high accuracy (94%) [114]. It seems that EUS elastography is better in RC staging than EUS alone especially for early cancers [115]. Contrast enhanced ultrasonography (CE-US) can be used to evaluate tumor vascularity and response to antiangiogenic treatment [116]. **(Figure 5)** Computed parameters can be used to quantify tumor angiogenesis and measure vascularity changes after therapy [117]. Finally, 3D-EUS development allows spatial display of rectal and perirectal anatomy [112]. **(Figure 6)** It improves accuracy for both T and N staging, better than EUS alone, especially in the middle third of the rectum [118]. Published data shows that its accuracy for N stage improves from 65% to 85% and for T stage is 97.1% for T1, 94.3% for T2, 95.7% for T3 and 98.5% for T4 [119-121].

批注 [CR9]: Figure 4 added in this resubmission in accordance to the comments of the reviewer 33116: eus elastography figure

批注 [CR10]: Figure 5 added in this resubmission in accordance to the comments of the reviewer 33116: eus contrast enhancement

批注 [CR11]: Figure 6 mentioned in the main text

COLON CANCER

Despite improvements in EUS technology that allows a forward viewing, the EUS examination of the colon has proved to be less accurate for T and N staging (81% and 52.4% respectively) [122]. This decrease is due to the difficulty in evaluating the proximal colon segments and bowel movement [123]. Mini-probe EUS can be passed through the working channel of regular colonoscopes and can be used to evaluate lesions of the entire colon compensating for some of these limitations [124].

ANAL CANCER

EUS is useful for assessing the involvement of anal sphincters in low rectal tumors and in the staging of anal squamous-cell carcinomas. Treatment

decisions in anal cancer depends on sphincter invasion and EUS has an accuracy of 96%, sensitivity of 100%, specificity of 87% and NPV of 100% in evaluating it [125, 126]. Clinical staging of anal cancer tends to under-diagnose sphincter invasion [127-129]. Most clinically classified T1-T2 patients will have T3 lesions under EUS evaluation [129]. Giovannini et al. confirm this in a prospective multicenter study and recommend that in T1-T2 N0 tumors, a transrectal EUS should be performed [130]. EUS can be used also to determine multimodality therapy response [131]. A greater proportion of T1–T2 N0 lesions classified by EUS had a complete response to treatment than those classified by conventional clinical staging (94.5% vs. 80%, respectively) [130]. The use of 3D-EUS in anal carcinoma seems to add some benefits in perirectal lymph node and tumor invasion detection, when compared to standard EUS, but further studies are needed [132].

批注 [CR12]: All references were edited and corrected in accordance to your suggestions

REFERENCES

批注 [CR13]: References were corrected in accordance to your comments

批注 [CR14]: References were corrected in accordance to your comments

批注 [CR15]: References were corrected in accordance to your comments

FIGURES LEGEND

Figure 1. Esophageal carcinoma staging by EUS T2 N1. The tumor is being measure (13.3 x 20.2 mm). It invades up to the muscularis propria (white arrow). A round, sharply demarcated and hypoechoic lymph node can be seen next to the tumor.

批注 [CR16]: More explanation of the figures were done in accordance to the reviewer 33116 comments

Figure 2. Gastric adenocarcinoma staging by EUS T3 N0. The tumor overcomes the muscularis propria (blue arrow) and penetrates the subserosal connective tissue (white arrow).

批注 [CR17]: More explanation of the figures were done in accordance to the reviewer 33116 comments

Figure 3. Rectal adenocarcinoma staging by EUS T4 N0. The tumor invasion overcomes the rectal wall and penetrates the prostate. There is a lack of separation plane between the tumor and the prostate (white arrow).

批注 [CR18]: More explanation of the figures were done in accordance to the reviewer 33116 comments

Figure 4. Shows a lymph node being evaluated by elastography, for a gastric tumor staging. A) Qualitative elastography (color tones red– green–blue) shows the lesion with a blue-predominant color tone, which represents a hard tissue and suggest malignancy. The Strain Ratio (quantitative elastography) is being calculated by compering two different areas (A and B). Area A includes as much of the target lesion as possible. Area B is selected within a soft (red) reference area outside the target lesion. The result (B/A= 141.7) suggests malignancy. B) Shows the round, sharply demarcated and hypoechoic lymph node (white arrow).

Figure 5. Shows the same lesion presented in figure 3 being evaluated by contrast enhanced ultrasonography (CE-US). The white arrow shows the lymph node with no enhancement after the contrast application, which suggest malignancy.

Figure 6. Rectal adenocarcinoma staging by 3D EUS T1 N1. The yellow arrows on the left show the muscularis propria. The tumor invades up to the submucosa. A white submucosa plane can be seen between the tumor (TU) and the muscularis propria. The yellow arrow on the right shows a round lymph node.

批注 [CR19]: More explanation of the figures were done and this figure was added in accordance to the reviewer 33116 comments

批注 [CR20]: More explanation of the figures were done and this figure was added in accordance to the reviewer 33116 comments

批注 [CR21]: More explanation of the figures were done in accordance to the reviewer 33116 comments