Endoscopic and Endobronchial Ultrasound-guided Needle Aspiration in the Mediastinal Staging of Non-small Cell Lung Cancer

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Abstract. *Invasive staging of mediastinal lymph nodes is recommended for the majority of patients with potentially resectable non-small cell lung cancer. In the past, 'blind' transbronchial needle aspiration during bronchoscopy and mediastinoscopy, a surgical procedure conducted under general anesthesia, were the only diagnostic methods. The latter is still considered the 'gold standard'; however, two novel, minimally-invasive techniques have emerged for the evaluation of the mediastinum: endoscopic (transesophageal) and endobronchial ultrasound – both performed using a dedicated echoendoscope, facilitating the ultrasound-guided, real-time aspiration of mediastinal lymph nodes. These methods are well-tolerated under local anesthesia and moderate sedation, with very low complication rates. Current guidelines on the invasive mediastinal staging of lung cancer still state that a negative needle aspiration result from these methods should be confirmed by mediastinoscopy. As more experience is gathered and echoendoscopes evolve, a thorough endosonographic evaluation of the mediastinum by both techniques, will obviate the need for surgical staging in the vast majority of patients and reduce the number of futile thoracotomies.*

Lung cancer is the most common cause of death from cancer worldwide (1). In the absence of distant metastasis, accurate staging of the mediastinum is crucial for patients with non-small cell lung cancer (NSCLC): it strongly influences prognosis and treatment of patients and facilitates the conduction of clinical trials by defining the N descriptor of the TNM staging system of lung cancer. Failure to correctly assess the mediastinal lymph node status can lead either to a futile thoracotomy (adding considerable morbidity to the patient) or to an unjustified exclusion of a patient from a potentially curative surgery in the case of a false-positive imaging test.

Non-invasive-imaging staging methods have evolved to incorporate functional information with the advent of Positron Emission Tomography/Computed Tomography (PET/CT) and their role in the initial evaluation of the mediastinum is established. However, they still lack accuracy and in most cases cannot perform as a stand-alone test. Darling *et al*. assessed the accuracy of PET/CT compared with invasive mediastinal staging by analyzing patients randomized to the PET/CT arm of the Early Lung PET trial (ELPET) who also underwent invasive staging (mediastinoscopy, thoracotomy or both) (2)). They reported positive and negative predictive values for PET of 64% and 95% respectively, stressing the need for pathologic confirmation of PET-positive lymph nodes. The American College of Chest Physicians guidelines of 2007 on mediastinal staging of NSCLC without distant metastases recommend invasive staging methods for the majority of patients with the exception of extensive mediastinal infiltration and peripheral clinical stage I tumor with negative PET in the mediastinum (3, 4).

There is a variety of invasive methods for the staging of the mediastinum. Transcervical mediastinoscopy is considered as the 'gold standard' procedure with the current guidelines stating that any non-diagnostic result from a needle-based technique should be confirmed by mediastinoscopy (3). Needle-based techniques are transthoracic needle aspiration (TTNA), transbronchial needle aspiration (TBNA), endoscopic ultrasound–guided fine-needle aspiration (EUS-FNA) and

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endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA). The latter two have gained popularity among clinicians and are being increasingly used, claiming to supersede surgical procedures such as mediastinoscopy. In this review, we focus on the existing evidence on the use of EUS-FNA, EBUS-TBNA and their combination in the invasive staging of the mediastinum in patients with NSCLC and their performance in comparison with mediastinoscopy.

EUS-FNA

Employment of an echoendoscope with a curvilinear ultrasound probe through the esophagus allows for visualization and real-time fine-needle aspiration of lymph nodes located in the posterior and inferior mediastinum [mainly lymph node stations 4L, 5, 7, 8 and 9 according to the Mountain-Dressler lymph node map (5). The left adrenal gland can also be sampled (when enlarged or PET-positive), thus offering complete staging and diagnosis in one sitting for some patients (6). This technique, initially used for pancreatic diseases, was first described for mediastinal node aspiration in 1993 (7) and is generally safe and well-tolerated under local anesthesia and conscious sedation. Current evidence on its diagnostic accuracy consists mainly of retrospective and prospective studies without randomization.

In a systematic review and meta-analysis of 2007, Micames *et al*. included two retrospective and 16 prospective studies (three with consecutive enrollment) as eligible studies, with a total of 1,201 patients included in the analysis (8). They reported a pooled sensitivity for EUS-FNA of 83% [95% confidence interval (CI)=78 to 87%] and a specificity of 97% (95% CI=96 to 98%), while even in patients without enlarged lymph nodes seen on CT scans, EUS-FNA was useful in sampling lymph nodes as small as 3 mm and prevention of futile surgery or mediastinoscopy though pooled sensitivity in this subgroup was lower (58%). Complications were minor and rare (0.8%).

In a small but well-balanced randomized control trial, Tournoy *et al*. compared surgical staging with EUS-FNA by allocating 40 patients with proven or suspected NSCLC without distant metastases either to surgical staging (n=21) or to EUS-FNA (n=19), followed by surgical staging in cases of a non-malignant result (9). EUS-FNA, with the assistance of rapid on-site evaluation (ROSE) by a cytopathologist, reduced the need for surgical staging by 68% (primary endpoint, $p<0.001$) and tended to have a higher but not statistically significant sensitivity (93 *vs*. 73%, $p=0.29$). Eloubeidi *et al*. confirmed malignant N2 or N3 lymph nodes with EUS-FNA in 13 of 35 (37%) patients who had already undergone mediastinoscopy that was negative for malignancy (10).

Lymph node features potentially predictive of malignancy were of round shape, sharp margins and short axis of more than 8.3 mm, in a prospective study of 425 patients (11), although their clinical importance is unknown. ROSE by a cytopathologist seems to increase its performance (12, 13) but relevant studies are very limited.

In conclusion, EUS-FNA is a valuable tool for lung cancer staging. However, limitations do exist: it has a modest negative predictive value – 78% (73% to 83%) in the meta-analysis by Micames *et al*. (8), thus a non-diagnostic result should be further evaluated. Moreover, the anterior mediastinum and the right paratracheal lymph node stations (2R, 4R) in particular cannot be accessed due to the interposed trachea. Lymph nodes of the aortopulmonary window are difficult but not impossible to sample by EUS-FNA.

EBUS-TBNA

Endobronchial ultrasound for the evaluation of the mediastinum and sampling of mediastinal lymph nodes is a novel diagnostic modality with increasing use in clinical practice. Initially, a radial ultrasound probe was employed through the working channel of a bronchoscope to visualize mediastinal lymphadenopathy for subsequent TBNA (14). First reports in 2004 on the use of a fiber optic bronchoscope, with the incorporation of a convex ultrasound probe on its tip to allow for real-time ultrasound-guided transbronchial needle aspiration with a dedicated 22-gauge needle, were very encouraging (15). The convex ultrasound transducer of 7.5 MHz scans to a depth of 50 mm, with an angle of view of 90˚ and a direction of view 30˚ forward obliquely. The needle can be visualized through the optics and the ultrasound (Figure 1).

In a prospective study of 70 selected patients with mediastinal or hilar lymphadenopathy of >1 cm, Yasufuku *et al*. reported excellent performance with sensitivity and specificity of 95.7% and 100% respectively (16). The procedure followed conventional flexible bronchoscopy and both were performed under local anesthesia and sedation. Surrounding blood vessels were confirmed using Doppler mode and no major complications occurred.

Herth *et al*. reported excellent diagnostic accuracy in a prospective study with consecutive enrollment of 502 patients referred for TBNA (17). The procedure was conducted under general anesthesia and jet ventilation in two-thirds of patients and local anesthesia in the rest, with a mean duration of 12.5 minutes for both groups. Results did not differ between the two groups. All patients had enlarged (>1 cm) mediastinal or hilar lymph nodes. Lymph node stations primarily accessible by EBUS-TBNA were those of the anterior-middle mediastinum (stations 2, 3, 4 and 7) and hilar (stations 10 and 11), with a mean size of punctured nodes of 1.6 cm. Sensitivity and specificity were 94% and 100%, respectively. No complications were reported.

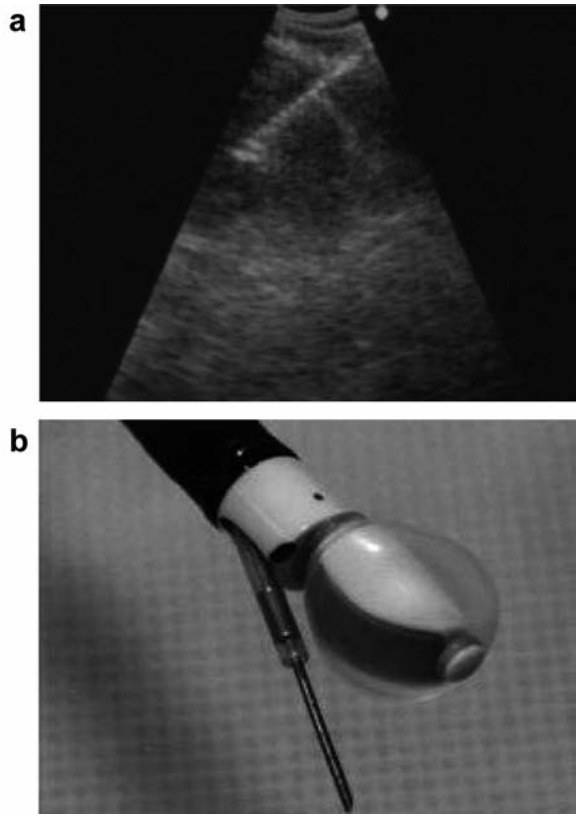

Figure 1. *A: Ultrasound image with real-time puncture of a subcarinal lymph node. B: Endobronchial ultrasound bronchoscope with inflatable ballon for maximization of contact with the bronchial wall and 22-gauge needle.*

In a prospective study of 102 potentially operable patients with diagnosed or suspected NSCLC, EBUS-TBNA was compared with PET and CT for lymph node staging of lung cancer (18). Both sensitivity and specificity were higher for EBUS-TBNA with a diagnostic accuracy of 98% for the procedure *vs*. 60.8% and 72.5% for CT and PET, respectively.

Performance of EBUS-TBNA in patients without enlarged mediastinal lymph nodes was evaluated in two studies from Heidelberg. In the first study in 2006, 100 patients with NSCLC and no lymph node >1 cm in the mediastinum on chest CT underwent EBUS-TBNA and subsequent surgical staging (19). A total of 119 lymph nodes, 5-10 mm in size, were punctured and 19 patients had positive findings for lymph node metastases despite a negative CT scan (16 patients with N2 or N3 disease). Additional positive nodes were detected by surgical staging in two patients. Sensitivity, specificity and negative predictive value for EBUS-TBNA were 92.3%, 100% and 96.3%, respectively. Prevalence of mediastinal metastases was 17%, similar to that reported in previous studies of surgical staging and negative mediastinal findings on chest CT. In the second study from the same team, patients with no enlarged lymph nodes on CT scans and negative PET in the mediastinum (defined as standardized uptake value <2.5) underwent EBUS-TBNA and subsequently surgical staging (mediastinoscopy or thoracotomy) (20). EBUS-TBNA detected disease-positive mediastinal lymph nodes in eight out of 97 studied patients and missed only one patient with N1 disease. Although the prevalence of mediastinal disease in this population was lower than in the previous study (19) (9% *vs*. 17%), owing probably to the higher sensitivity of PET compared with CT, the negative predictive value of EBUS-TBNA remained as high as 96.3%. Concluding, the authors suggested that perhaps a minimally-invasive and safe procedure such as EBUS-TBNA should be considered for the preoperative staging of patients with NSCLC even in the absence of mediastinal disease in the imaging tests.

Consecutive patients with potentially resectable NSCLC were enrolled in a large study by Szlubowski *et al*. (21) regardless of mediastinal status on chest CT. They all underwent EBUS-TBNA and in those with negative results, transcervical extended bilateral mediastinal lymphadenectomy (TEMLA) was performed. The majority of patients had N2 lymph nodes enlarged on CT scans (184 patients out of 226 included). Overall sensitivity and negative predictive value were 89 and 83.5% respectively with false negative results in 16 patients (5.3%), 12 of whom had positive lymph nodes in stations accessible with EBUS-TBNA. It bears notice, however, that a more accurate confirmatory test such as TEMLA was used instead of standard cervical meidastinoscopy.

In a prospective, crossover trial Ernst *et al*. compared EBUS-TBNA with standard cervical mediastinoscopy in 66 patients with mediastinal adenopathy to lymph node stations 2, 4, or 7 who underwent both procedures (22). Surgical lymph node dissection was used as the accepted standard. In these selected patients with either or both paratracheal and subcarinal enlarged nodes, diagnostic yield of EBUS-TBNA was superior with sensitivity, specificity and negative predictive value of 87%, 100% and 78% respectively *versus* 68%, 100% and 59%, respectively, for mediastinoscopy. This higher diagnostic yield for EBUS-TBNA compared with mediastinoscopy (91% *versus* 78% respectively; p=0.007) was attributed to the better performance for subcarinal lymph nodes, probably due to the better access to the posterior subcarinal station, while there was no difference in the paratracheal lymph nodes.

In a more recent and large prospective controlled trial comparing EBUS-TBNA and mediastinoscopy, both procedures were performed in the same setting by a thoracic surgeon in patients with suspected or proven NSCLC who would otherwise need mediastinoscopy for preoperative staging (23). ROSE was used for the adequacy of EBUS-TBNA specimens but its results were blinded to the surgeon

performing the subsequent mediastinoscopy. A total of 153 patients were analyzed and paratracheal and subcarinal lymph nodes were mainly sampled. Both procedures had similar diagnostic performance, with sensitivity, negative predictive value and accuracy for EBUS-TBNA of 81%, 91% and 93%, respectively, and for mediastinoscopy of 79%, 90% and 93%, respectively. No complications were reported for EBUS-TBNA, while minor complications were observed in four patients (2.6%) from mediastinoscopy.

EBUS-TBNA is also very useful in the diagnosis of isolated mediastinal lymphadenopathy, obviating the need for mediastinoscopy in 87% of cases (24).

The use of ROSE seems to further improve performance and reduce the number of needle passes of EBUS-TBNA(25, 26) although there are contradictory reports (27) and further studies are needed. No more than three needle aspirations are needed, while in the presence of a tissue core specimen even as few as two aspirations are enough (28). Suction applied to the needle during sampling of lymph nodes probably does not increase adequacy of specimens (29).

In cases where both diagnosis and staging of lung cancer are achieved by EBUS-TBNA, it is essential that it can provide enough material for subtyping and genotyping of NSCLC. In a large multicenter study of 774 patients with known or suspected NSCLC, cytological specimens obtained by EBUS-TBNA enabled subtyping in 77% of patients, while the rate of NSCLC not otherwise specified (NSCLC-NOS) was reduced by half with the use of immunohistochemistry (30). Epidermal growth factor receptor (EGFR) mutation status was assessed in 90% of cases. Molecular profiling of NSCLC with mutation analysis of the *EGFR*, *KRAS* oncogene, *B-Raf* proto-oncogene and phosphatidylinositol 3-kinase catalytic subunit-alpha (*PIK3CA*) was performed successfully in a series of 43 patients on fine needle cytological aspirates (31) using allele-specific real-time polymerase chain reaction (*qPCR*). EBUS-TBNA aspirates routinely prepared were sufficient for *EGFR* and *KRAS* mutation testing in 96% of cases in a study comprising of several cytological specimens from patients with lung cancer (32). However, specimen representativeness in mixed tumors as well as possible discrepancies in molecular features (*i.e. EGFR* mutation status) between the primary tumor and local lymph node metastases (33, 34) may have clinical implications, especially when NSCLC is diagnosed solely by a cytological specimen of EBUS-TBNA.

Finally, the use of both EBUS-TBNA and EUS-FNA in the setting of re-staging of the mediastinum after induction therapy in patients with stage IIIA NSCLC has not been studied extensively. However, in a systematic review by Candela and Detterbeck (35), the average false-negative rate of needle techniques (EBUS-TBNA, EUS-FNA and TBNA) compared with repeat mediastinoscopy was 14% *versus* 22%, respectively. Moreover, when initial staging of these patients is conducted with minimally invasive procedures, primary mediastinoscopy may be preserved for restaging of the mediastinum.

The Combined Approach

EBUS and EUS provide complementary access to the mediastinum and their combined use, termed also as complete 'medical' mediastinoscopy (36), may obviate the need for surgical staging in potentially resectable NSCLC. EBUS-TBNA can sample lymph nodes of the anterior mediastinum (similarly to cervical mediastinoscopy) and hila. EUS-FNA can access the posterior and inferior mediastinum (Table I).

Searching the literature (MEDLINE) up to January of 2013, eight studies on the combined use of EBUS and EUS were found (37-44), with only one randomized controlled trial among them (41).

First reports on the combined endosonographic evaluation of the mediastinum in 2005 consisted of small series of patients. Vilmann *et al*. studied 33 patients either diagnosed with NSCLC, or with mediastinal lesion suspicious of lung cancer, who underwent both procedures (38). In a total of 119 lesions sampled, EBUS-TBNA added 11 cancer diagnoses to EUS-FNA samples and, conversely, EUS-FNA revealed 12 samples with metastasis missed by EBUS-TBNA. Combined, the two procedures had excellent performance. In another small study of 20 selected patients who underwent EBUS-TBNA, when added to seven of them, EUS-FNA contributed information in all cases (37).

In 2008, Wallace *et al*. compared the accuracy of TBNA, EBUS-TBNA and EUS-FNA by performing all procedures sequentially in 138 patients with suspected lung cancer (39). Histology or adequate clinical follow-up (6 to 12 months) were used as a reference standard. The combined EBUS-TBNA and EUS-FNA had higher sensitivity (93%) and negative predictive value (97%) than either method alone for any mediastinal location and even in patients with normal mediastinum on chest CT scans. Each method individually detected 29 out of the 42 patients with malignant lymph nodes, but combined, they detected 39 out of these 42 patients. Blind TBNA was positive in only 15 patients.

Hwangbo *et al*. (40) and Herth *et al*. (42) published simultaneously on combined endobronchial and transesophageal ultrasound-guided FNA with the use of one ultrasound bronchoscope for both procedures. Both studies confirmed the feasibility of this practical approach. Endoscopic ultrasound with bronchoscope-guided (EUS-B) - FNA, a term introduced by Hwangbo *et al*., was used as an add-on procedure for mediastinal nodes inaccessible to previously performed EBUS-TBNA. EUS-B-FNA detected three additional patients to the 38 detected by EBUS-TBNA (out of 150 studied) with mediastinal metastases, although

Table I. *Lymph node access of different staging modalities. *Aortopulmonary window (5) and para-aortic (6) lymph nodes are difficult to access by conventional methods and extended cervical mediastinoscopy; Chamberlain procedure or video-assisted thoracoscopic surgery may be required. Endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA) can occasionally sample these lymph nodes (45), while there are reports (46), on the transaortic puncture of para-aortic lymph nodes (station 6).*

Lymph node station	2R	2L	4R	4L	5*	6*	7	8	9	10, 11
EBUS-TBNA	+	+	+	+	-	-	+	-	-	+
EUS-FNA	-	-	-	+	±	±	+	+	+	-
Mediastinoscopy	+	+	+	+	-	-	+	-	-	-
Combined EBUS-EUS	+	+	+	+	±	±	+	+	+	+

EBUS-TBNA: Endobronchial ultrasound-guided transbronchial needle aspiration.

differences in sensitivity and diagnostic accuracy (additional diagnostic gain 6.7%) were not statistically significant for the combination. However, the proportion of accessible mediastinal lymph nodes increased significantly with the addition of EUS-B-FNA to EBUS-TBNA from 78.6% to 84.6% (p=0.015). Herth *et al*. studied 150 consecutive patients with suspected NCLC and reported sensitivity for EBUS, EUS and for the combined approach with a single ultrasound bronchoscope of 92%, 89% and 96%, respectively (42).

In a group of 120 consecutive patients with NSCLC with no enlarged mediastinal lymph nodes seen on CT scans (clinical stage IA-IIB), Szlubowski *et al*. performed combined EUS-FNA and EBUS-TBNA and, when both were negative for malignancy, bilateral TEMLA was conducted (44). Thoracotomy with appropriate pulmonary resection and systematic mediastinal lymph node dissection followed a negative TEMLA. The prevalence of mediastinal lymph node metastases was quite low, 22%, as would be expected for the radiologically-normal medistinum. Sensitivity and negative predictive value were 46% and 86%, respectively, for EBUS-TBNA, 50% and 87%, respectively, for EUS-FNA, and 68% and 91%, respectively, for the combination. The superiority of the combined approach was statistically significant.

Ohnishi *et al*. compared the combined endoscopic evaluation of mediastinum with PET-CT in a series of 120 consecutive patients with suspected resectable lung cancer (43). EUS-FNA combined with EBUS-TBNA were significantly superior than PET-CT yielding diagnostic accuracy of 90% *vs*. 73.6% respectively (p<0.0001).

Annema *et al*., in the only randomized controlled multicenter trial conducted so far, addressed the issue of direct comparison of combined endosonography with cervical mediastiniscopy, the 'gold standard' for the mediastinal staging of NSCLC (41). They randomized 241 consecutive patients with potentially resectable NSCLC and indication for invasive mediastinal staging according to current guidelines (3, 4), either to surgical staging by mediastinoscopy (n=118) or to endosonography with the combined EUS-FNA and EBUS-TBNA (n=123), followed by surgical staging in cases with a negative needle result (65 out of 123 patients) – as dictated by current guidelines (3). In the absence of N2/N3 disease or direct mediastinal tumor invasion, thoracotomy was performed. The primary end-point was sensitivity for mediastinal metastases. The combined endoscopic approach plus surgical staging resulted in a superior diagnostic performance, with sensitivity of 94% *vs*. 79% for surgical staging alone and a negative predictive value of 93% *vs*. 86%, respectively. The number of futile thoracotomies was 21 out of 118 patients (18%) in the surgical staging group *vs*. 9 out of 123 patients (7%, p=0.02) in the endosonography-plus-surgical staging group. The rate of complications (5-6%) was similar in the two groups; however, most complications (12 out of 13) were directly related to surgical procedures. Endosonography alone without surgical staging for negative results exhibited comparable sensitivity and negative predictive value to mediastinoscopy (85% and 85% *vs*. 79% and 86%, respectively), with a much lower rate of complications (1% *vs*. 6%, p=0.03). Most importantly, surgical staging following negative endosonography detected six out of 65 patients (9%) with mediastinal metastases, meaning that after a negative, minimally-invasive endoscopic staging of the mediastinum, 11 patients would have to undergo surgical staging in order to avoid one unnecessary thoracotomy according to current guidelines on invasive staging of the mediastinum in patiens with NSCLC (3).

Table II summarizes the basic characteristics of the most important studies on the performance of combined EUS-FNA and EBUS-TBNA.

Conclusion

EUS-FNA and EBUS-TBNA are both safe, minimally-invasive procedures for the mediastinal staging of lung cancer. Diagnostic performance of these techniques is excellent when performed by experienced endoscopists. Their access to the mediastinum is complementary and, when combined, they yield higher sensitivity for the detection of mediastinal

Table II. *Performance of the combined approach of endoscopic and endobronchial ultrasound (CUS) for the mediastinal staging of NSCLC.*

Study/year	Type	No. of patients	Sensitivity	Specificity	NPV	Accuracy	Prevalence of N2/N3 disease
Wallace *et al*. (39)/2008	Consecutive patients	138	93%	97%	97%		30% (42/138)
Hwangbo *et al*. (40)/2010	Consecutive patients	150	91.1%	100%	96.1%	97.2%	30% (45/150)
Herth *et al*. (42)/2010	Consecutive patients	150	96%	100%	95%	100%	51% (71/139)
Szlubowski *et al*. (44)/2010	Consecutive patients	120	68%	98%	91%	91%	22% (Radiologically normal mediastinum)
Ohnishi *et al*. (43)/2011	Consecutive patients	120	71.8%	100%	86.6%		
Annema *et al*. (41)/2010	Randomized controlled trial	241	85% CUS alone 94% CUS+surgical		85% CUS alone 93% CUS+surgical		49%

NPV: Negative predictive value.

metastases than does mediastinoscopy, thus reducing the number of futile thoracotomies. It is possible that the use of a single echoendoscope could increase the implementation of the combined approach by respiratory specialists. More randomized trials are needed to clarify the necessity for surgical staging after a negative complete endosonographic evaluation of the mediastinum.

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