Universitat Autònoma de Barcelona

La Ultrasonografia Endobronquial en l'estudi del mediastí neoplàsic

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TESI DOCTORAL 2018.

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Universitat Autònoma de Barcelona

Els Doctors Felipe Andreo Garcia i Jose Sanz Santos certifiquen que la Tesi Doctoral titulada **"*La Ultrasonografia Endobronquial en l'estudi del mediastí neoplàsic*"** presentada per Pere Serra Mitjà, sota la seva direcció, compleix les exigències metodològiques i científiques requerides i es considera apta per a ser defensada pel Doctorat en Medicina de la Universitat Autònoma de Barcelona.

Per tal que quedi constància, es signa aquest document a Badalona, a 6 de Setembre de 2018.

Felipe Andreo Garcia Jose Sanz Santos

El Doctor Juan Ruiz Manzano certifica que la Tesi Doctoral titulada **"*La Ultrasonografia Endobronquial en l'estudi del mediastí neoplàsic*"** presentada per Pere Serra Mitjà, de la que n'és tutor, compleix les exigències metodològiques i científiques requerides i es considera apta per a ser defensada pel Doctorat en Medicina de la Universitat Autònoma de Barcelona.

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Juan Ruiz Manzano

A vosaltres, Pares.

A tu, Maria.

A tu, Blai.

Abreviatures

CP	Càncer de pulmó.
CPNCP	Carcinoma Pulmonar no cèl·lula petita.
EGFR	Receptor del factor de creixement epidèrmic.
EML4-ALK	Fusió de gens de la quinasa de limfoma anaplàstic.
ESTS	de l'anglès, European Society of Thoracic Surgeons.
GATA-3	de l'anglès, GATA binding protein 3.
HER2	Receptor del factor de creixement epidèrmic humà
PATB	Punció Aspirativa Transbronquial.
PD-L1	de l'anglès, Programed Death-ligand 1.
PET	Tomografia per emissió de positrons.
PET-TC	Tomografia per emissió de positrons amb Tomografia computeritzada.
PIK3CA	Gen de la subunitat catalítica de la fosfatidilinositol 3-quinasa.
PTT	Punció transtraqueal.
RE	Receptors d'Estròg ens.
RM	Ressonància magnètica.
ROSE	de l'anglès, Rapid on-site evaluation.
ROS-1	Proto-oncogen ROS-1.
RP	Receptors de Progesterona.
SBRT	Radioteràpia corporal estereotàctica.
TB	Tuberculosi
TC	Tomografia computeritzada.
TNM	Tumor, gangli, metàstasi.
TTF-1	de l'anglès, Thyroid transcription factor-1
USE	Ultrasonografia Endoscòpica.
USE-B	Ultrasonografia Endoscòpica amb ecobroncoscopi
USE-PA	Ultrasonografia Endoscòpica amb punció aspirativa.
USE-B-PA	Ultrasonografia Endoscòpica amb ecobroncoscopi amb Punció Aspirativa.
USEB-PATB	Ultrasonografia Endobronquial amb Punció Aspirativa Transbronquial.
VPN	Valor predictiu negatiu.
VPP	Valor predictiu positiu.

Taula de continguts

Resum

RESUM

La present Tesi Doctoral es basa en tres articles que avaluen la idoneïtat de la ultrasonografia endobronquial amb punció aspirativa transbronquial (USEB-PATB) en l'estudi del mediastí neoplàsic, tant d'origen pulmonar com extra-pulmonar.

En aquests estudis, la USEB-PATB ha demostrat la seva utilitat en l'estudi del mediastí patològic, tant en l'estadiatge del càncer de pulmó (CP) com en l'estudi de la recidiva a nivell ganglionar. En l'estadiatge, la realització de la USEB-PATB de forma sistemàtica en pacients amb càncer de pulmó no cèl·lula petita (CPNCP) i afectació mediastínica ganglionar ha demostrat aportar més informació clínica vers l'estadiatge dirigit a la Tomografia per emissió de positrons amb Tomografia computeritzada (PET-TC), i en l'estudi de la recidiva local de pacients amb càncer de pulmó tractat quirúrgicament, ha demostrat ser un procediment precís pel diagnòstic, i més, unit a l'Ultrasonografia Endoscòpica amb Ecobroncoscopi amb punció aspirativa (USE-B-PA).
L'obtenció de mostres ganglionars mitjançant la USEB-PATB ha demostrat ser òptima per a realitzar estudis que permetin la identificació de mutacions en pacients amb metàstasi mediastíniques de càncers d'origen extra-pulmonar, com la identificació dels Receptors d'Estròg ens (RE), Receptors de Progesterona (RP) i l'expressió del receptor del factor de creixement epidèrmic humà (HER2) en pacients amb metàstasi mediastíniques de càncer de mama.

La USEB-PATB ha demostrat ser una tècnica mínimament invasiva, segura i útil per l'estudi del mediastí afecte per malaltia neoplàsica.

SUMMARY

The present doctoral thesis is based on three articles that evaluate the accuracy of endobronchial ultrasonography with transbronchial needle aspiration in the study of the mediastinum, both pulmonary and extra-pulmonary origin.

In these studies, endobronquial ultrasound with transbronchial needle aspiration has shown its usefulness in the study of abnormal mediastinum, both in the nodal staging of lung cancer and in the diagnosis of locoregional recurrence.
In nodal staging, performing systematic endobronquial ultrasound with transbronchial needle aspiration in patients with non-small cell lung cancer and mediastinal nodal involvement has shown to provide more relevant clinical information than targeted staging. In patients with surgically resected NSCLC, EBUS-TBNA has demonstrated to be a precise procedure for the diagnosis of recurrence, especially when combined with EUS-B-NA.

Moreover, samples obtained by means of endobronchial ultrasound with transbronchial needle aspiration has demonstrated to be optimal for the identification of molecular mutations in patients with mediastinal metastases of extra-pulmonary cancers, as it would be the identification of the Estrogen Receptors, Progesterone Receptors and the expression of the human epidermal growth factor receptor in patients with mediastinal metastases of breast cancer.

In summary, EBUS-TBNA is a minimally invasive, safe and useful technique for the study of mediastinal affection for neoplastic disease.

Justificació

El càncer de pulmó és una de les malalties amb major mortalitat del món, amb un laboriós procés diagnòstic, i sovint presenta afectació ganglionar mediastínica algun moment de la seva evolució. A més, si tenim present que càncers d'altres etiologies poden afectar al mediastí, entenem que l'estudi del mediastí en pacients amb malaltia neoplàsica, en procés diagnòstic o ja tractada, és d'una importància cabdal.

La USEB-PATB és una tècnica ja ben establerta que permet l'estudi del mediastí d'una forma segura i acurada, havent demostrat la seva utilitat en el diagnòstic i estadiatge del càncer de pulmó, així com en l'estudi de recidives a nivell ganglionar, en el re-estadiatge i en el diagnòstic de neoplàsies no pulmonars, mitjançant la publicació de múltiples estudis fins el moment actual.

S'ha intentat descriure la millor manera de realitzar la tècnica o d'obtenir el mínim de mostres per que sigui valorable, en comparació amb el PET-TC però no s'havia demostrat fins ara que fer-ho de forma sistemàtica fos la forma més precisa.

Alguns estudis previs han demostrat la utilitat de USEB-PATB en el diagnòstic de recidiva ganglionar en pacients amb càncer de pulmó prèviament tractat però no disposem de series llargues de pacients ni de series només de pacients prèviament tractats, ni s'havia descrit la addició de la USE-B-PA.

La USEB-PATB ja havia demostrat ser un procediment precís pel diagnòstic de metàstasi ganglionars de tumors extra-toràcics, obtenint mostres adequades per a l'anàlisi molecular, però no s'havia avaluat fins el moment els estats d'expressió de diferents receptors específics de pacient amb càncer de mama, ni s'havia demostrat que poden portar a l'elecció d'un o altre tractament pels pacients.

Per tant, l'objectiu dels treballs que conformen la present Tesi Doctoral és demostrar la utilitat de la USEB-PATB en l'estudi del mediastí en pacients amb malaltia neoplàsica amb afectació mediastínica, així com en el diagnòstic de recidiva ganglionar i la utilitat de les mostres obtingudes per l'estudi molecular dels pacients amb càncer de mama.

Introducció

El Mediastí.

1. Descripció del mediastí.

El mediastí es defineix de forma genèrica com l'espai existent entre els dos sacs pleurals. S'estén des de l'estèrnum, a la part anterior, fins la columna vertebral, i des de l'obertura superior del tòrax fins el diafragma caudalment. Es divideix en dues parts: mediastí superior i inferior, que alhora, es subdivideix en mediastí anterior, mig i posterior. La separació entre el mediastí superior i inferior es basa en un pla que passa a nivell de l'articulació manubri-esternal (ventralment) i de la cara inferior de la IV vèrtebra toràcica (dorsalment)[1].

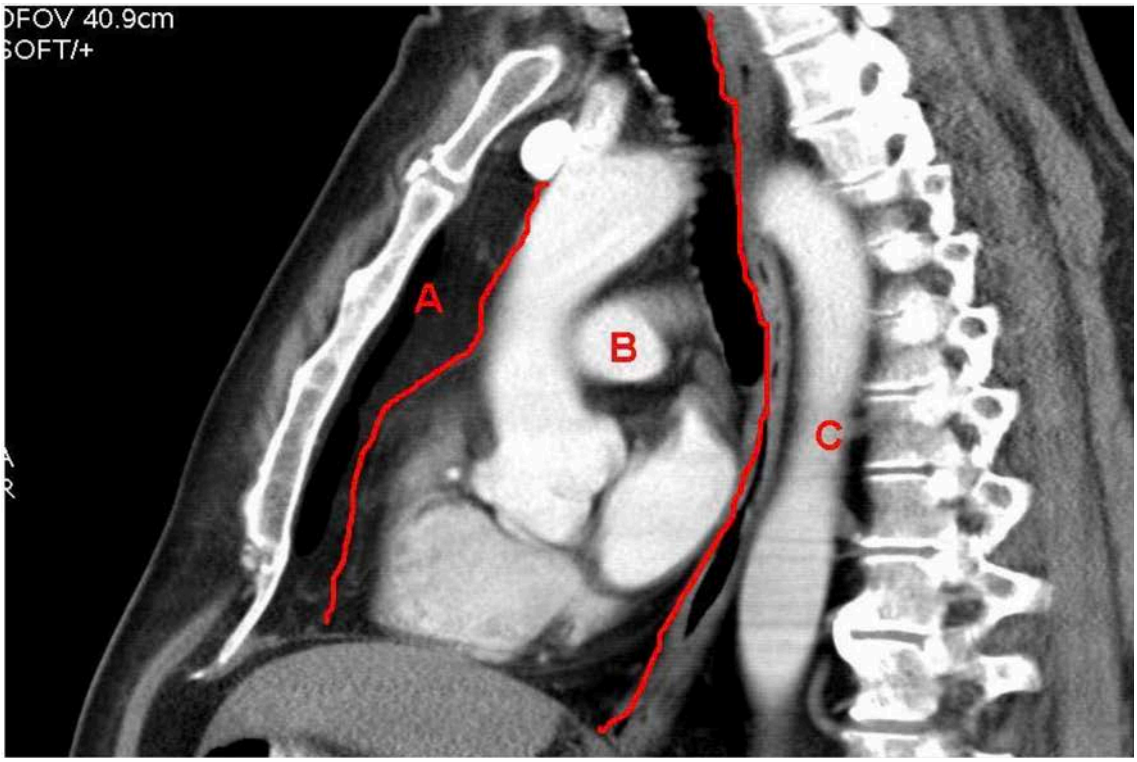

Figura 1. Imatge obliqua de TC que mostra les subdivisions del mediastí: anterior (A), mig (B) i posterior (C).

El mediastí superior conté l'origen dels músculs esternohioideu i esternotiroideu i l'extrem inferior del múscul llarg del coll, la crossa de l'aorta, el tronc arterial braquicefàlic, les arteries caròtides primitiva i subclàvia esquerra, els troncs

venosos braquicefàlics i la meitat superior de la vena cava superior, la vena intercostal superior esquerra, els nervis vague, cardíac, frènic i laringi recurrent esquerre, i la porció superficial del plexe cardíac, la tràquea, l'esòfag, el conducte toràcic, les restes del timus, i els ganglis limfàtics paratraqueals, braquicefàlics i alguns dels traqueobronquials.

El mediastí anterior conté teixit areolar, els lligaments esternopericàrdics, dos o tres ganglis limfàtics, branques mediastíniques de l'artèria mamària interna i de vegades part o restes tímiques.

El mediastí mig, la part més àmplia del mediastí inferior, conté el cor, el pericardi, l'aorta ascendent, la meitat inferior de la vena cava superior, la part terminal de la vena àziga, la bifurcació traqueal, els dos bronquis, l'artèria pulmonar i la seva divisió en les branques dreta i esquerra, les venes pulmonars dretes i esquerres, els nervis frènics, la part profunda del plexe cardíac i alguns ganglis limfàtics traqueobronquials.

Al mediastí posterior hi trobem la porció descendent de l'aorta toràcica, les venes àziga i hemiàziga, els nervis vagues i esplàncnics, l'esòfag, el conducte toràcic i els ganglis limfàtics mediastínics posteriors.

2. Tumors del mediastí.

La subdivisió del mediastí en els diferents compartiments és d'especial interès pel diagnòstic de lesions mediastíniques, atès que cadascun conté estructures anatòmiques pròpies[2]. La probabilitat de malignitat de les lesions, està influenciada principalment pels tres factors següents: localització de la lesió, edat del pacient i la presència o absència de símptomes. Encara que més de dos terços dels tumors mediastínics són benignes, les masses del compartiment anterior són més propensos a ser malignes[3].

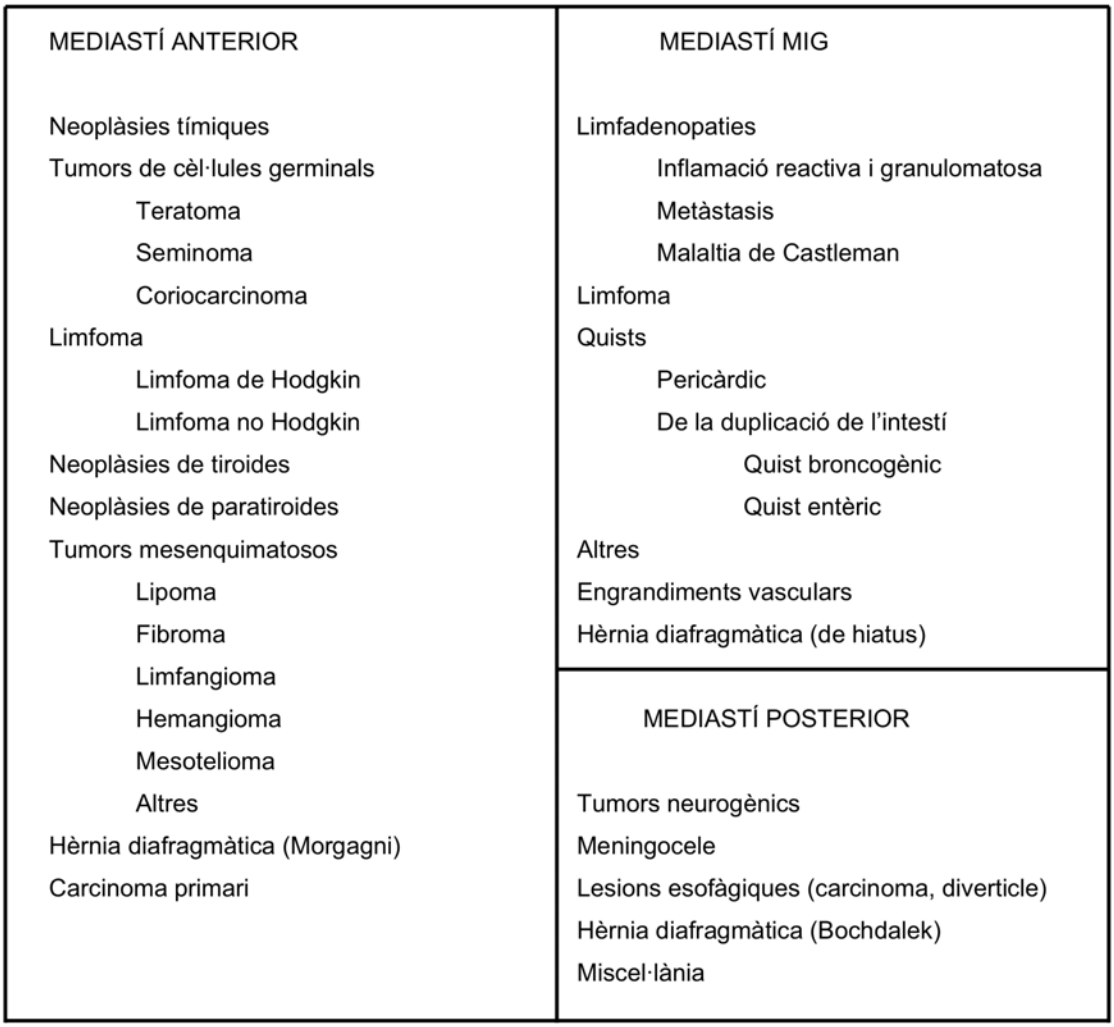

Figura 2. Classificació de les lesions mediastíniques.

Les masses mediastíniques són relativament poc freqüents i inclouen una gran varietat de diagnòstics diferencials entre neoplàsies, alteracions congènits, vasculars, etc. Per tant, és molt important l'estandardització d'aquestes subdivisions per definir al màxim el diagnòstic amb proves d'imatge i minimitzat les proves invasives.

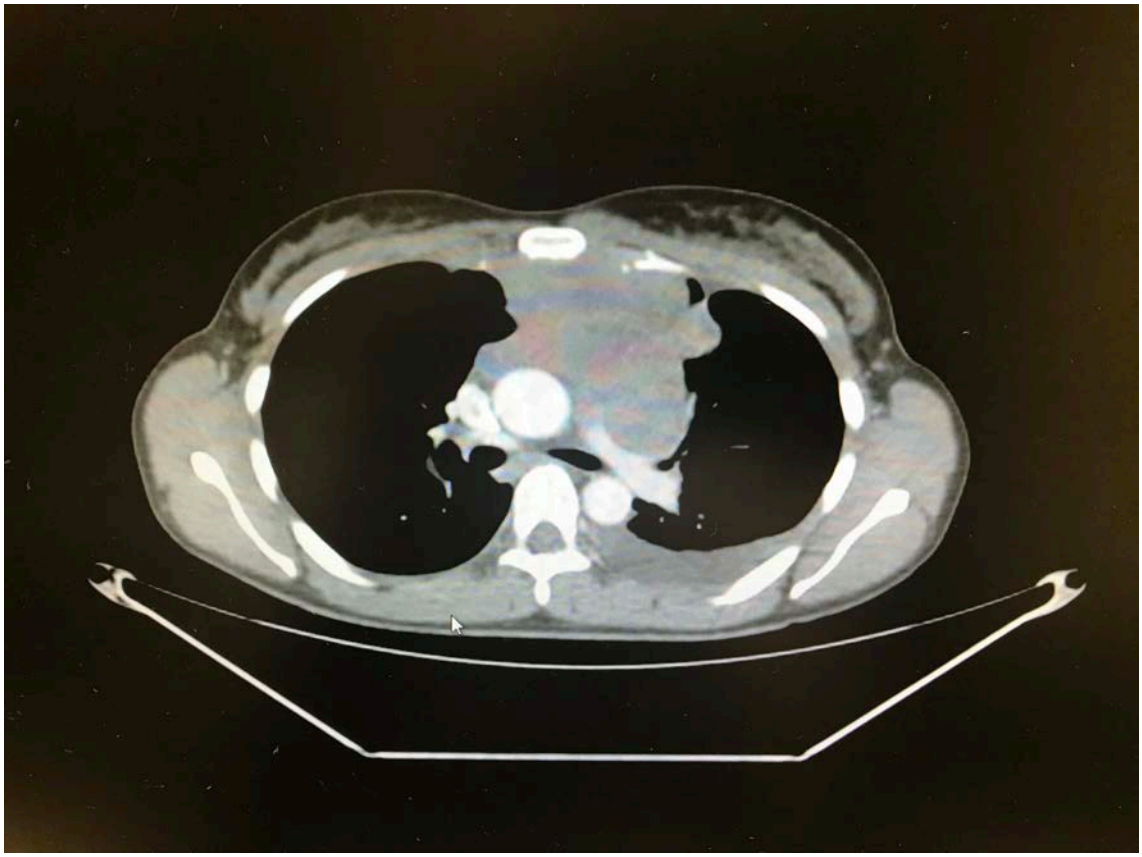

Figura 3. Imatge TC de massa mediastínica anterior.

3. Tècniques d'estudi del mediastí.

La primera prova d'imatge a realitzar davant la sospita d'una massa mediastínica és una radiografia simple de tòrax (front i perfil). Tot i això, la TC és la prova d'elecció en aquest cas. També la Ressonància magnètica (RM) és una prova usada, però tot i els avenços en l'obtenció d'imatges de RM, que han donat lloc a una millor qualitat d'imatge i a un menor temps d'adquisició, la imatge de RM es fa servir en gran mesura com a complement de l'escaneig de TC en l'avaluació del mediastí[4], agafant importància en la diferenciació de lesions quístiques o en pacients amb contraindicació d'administració de contrast. També és de molta utilitat la realització del PET per a l'estudi del mediastí que és una tècnica de medicina nuclear que permet discriminar les lesions en funció de la seva activitat metabòlica. La introducció d'escàners combinats de TC amb PET (PET-TC) permet l'adquisició gairebé simultània de les imatges, obtenint així imatges òptimes de fusió, sent actualment el mètode més avançat per a la imatge metabòlica i és capaç de

localitzar i avaluar els tumors de forma precisa. La utilització combinada redueix falsos positius i millora la fiabilitat en l'estadiatge del CP[5].

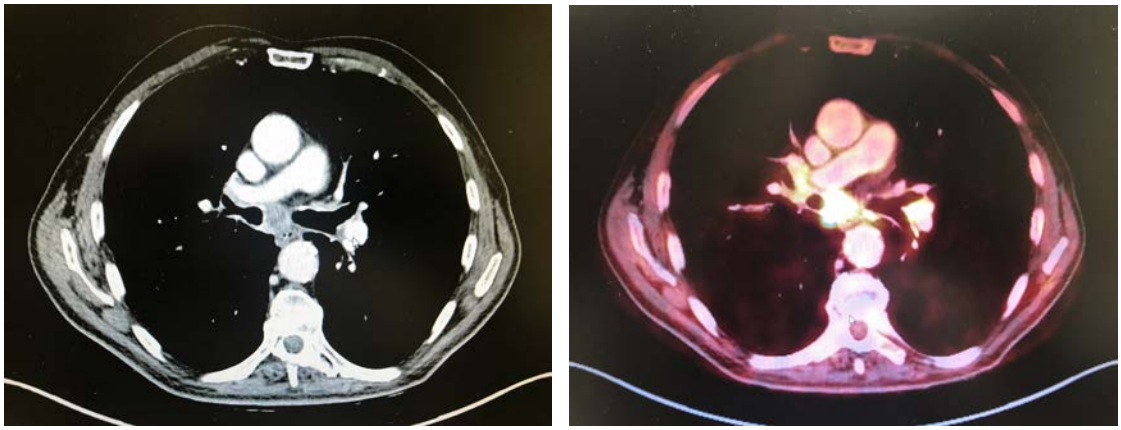

Figura 4. Imatges de TC i PET-TC d'adenopatia subcarinal.

Malgrat els importants avenços que han aparegut al camp de les tècniques d'imatge pel diagnòstic de metàstasi ganglionars mediastíniques, les mostres citològiques i/o histològiques continuen essent fonamentals en la gran majoria de lesions mediastíniques dels pacients, i en aquest sentit, disposem d'un ampli ventall de tècniques endoscòpiques i quirúrgiques.

Dins les tècniques endoscòpiques disposem de la punció transtraqueal (PTT) i la punció transbronquial (PTB), la ultrasonografia endoscòpica (USE) i la Ultrasonografia endobronquial (USEB). Les tècniques quirúrgiques són la mediastinoscòpia cervical, la mediastinotomia, la mediastinoscòpia cervical ampliada, la limfadenectomia mediastínica guiada per vídeo (VAMLA), la limfadenectomia mediastínica transcervical ampliada (TEMLA) i la toracoscòpia.

Ultrasonografia Endobronquial amb Punció Aspirativa Transtraqueal - Transbronquial (USEB-PATB).

1. La ultrasonografia endobronquial:

Hürter i Hanrath van ser els primers en descriure la ultrasonografia endobronquial (USEB) l'any 1992[6] mitjançant la inserció d'una sonda d'ultrasonografia a través del canal de treball d'un broncoscopi flexible. Això va portar a la creació de sondes d'ultrasonografia que s'inserien dins del canal de treball d'un broncoscopi flexible (les primeres amb baló per l'estudi de lesions central i posteriorment sense per l'estudi de lesions perifèriques) i més tard, a la fabricació de broncoscopis amb sondes ecogràfiques a l'extrem distal que avui coneixem com a ecobroncoscopis i permeten la realització de punció aspirativa transbronquial (PATB).

L'objectiu del broncoscopi lineal va ser aconseguir realitzar una PATB en temps real sota control ecogràfic. El primer sistema de ultrasonografia endobronquial amb punció aspirativa transbronquial (USEB-PATB) es va descriure el 2004[7].

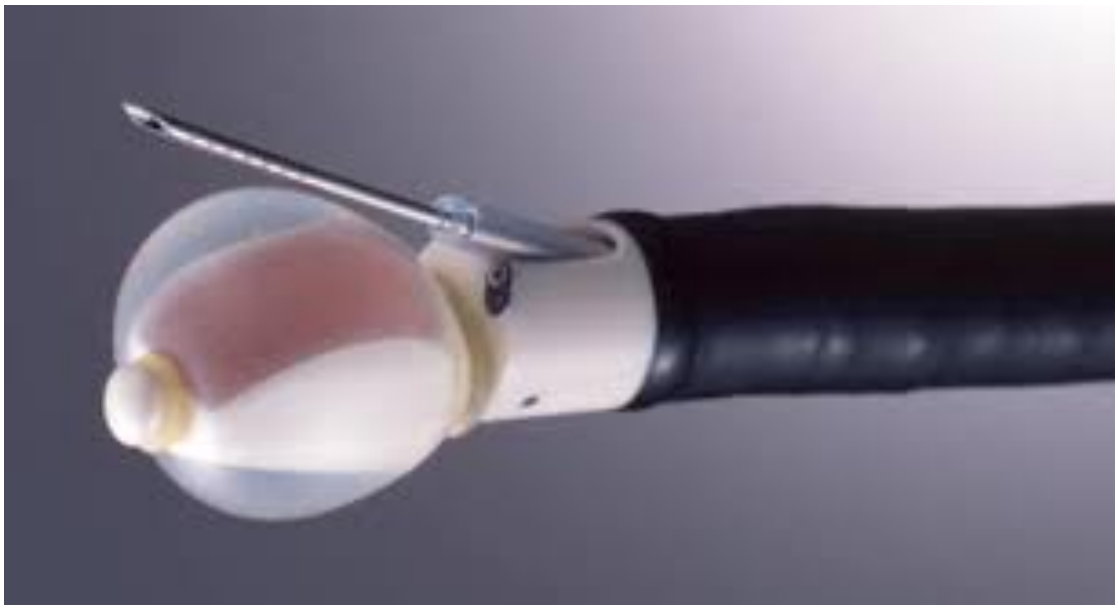

Figura 5. Ecobroncoscopi BF-UC160F-OL5.

En l'actualitat es disposen de dos tipus d'USEB: La USEB amb sonda radial i la USEB amb sonda lineal o sectorial.

La USEB radial, utilitza un transductor rotacional a la punta de la sona d'ultrasonografia, que s'introdueix a través del canal de treball del broncoscopi i que produeix una imatge de 360º al llarg de l'eix del broncoscopi per la visualització de les estructures peribronquials, fent servir altes freqüències (20-30MHz). La seva utilitat se centra en la detecció de lesions pulmonars perifèriques i estudis de la paret bronquial i vies aèries centrals en funció de la mida de la sonda radial.

Figura 6. (a) Sonda Radial; (b) Visió ecogràfica radial d'una lesió pulmonar.

La USEB lineal o sectorial utilitza un transductor convex situat a la punta de l'ecobroncoscopi flexible, que genera ultrasons de baixa freqüència (5-10MHz) que genera una imatge de sectorial de les estructures peribronquials en temps real. Permeten major penetració amb menys resolució, per visualitzar les estructures que hi ha més enllà de la paret bronquial. Disposa de doppler color per la visualització de les estructures vasculars. La seva utilitat es centra en la visualització de les estructures mediastíniques i poder realitza una punció aspirativa transbronquial guiada per ultrasonografia endobronquial (USEB-PATB) en temps real. El seu ús es va descriure el 2004[8], demostrant i un gran potencial per la visualització dels ganglis mediastínics.

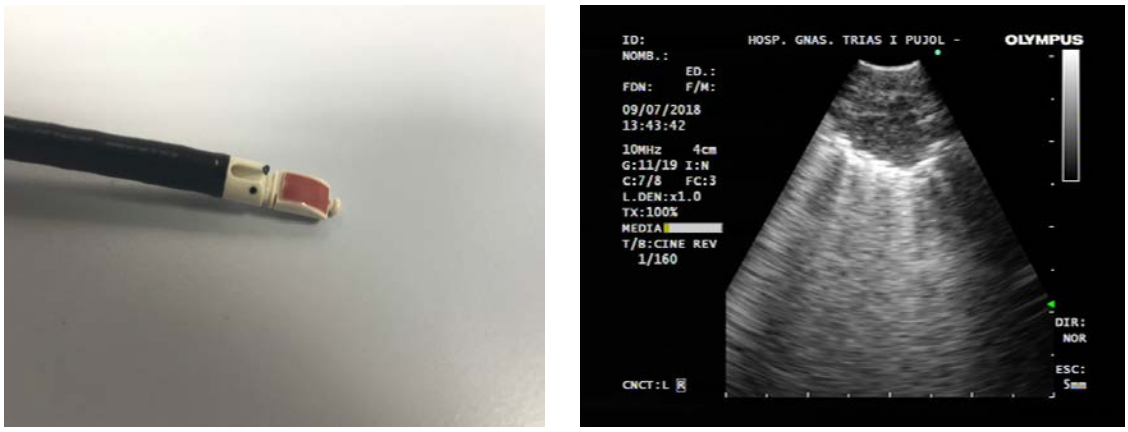

Figura 7. (a) USEB lineal; (b) Imatge ecogràfica d'una lesió mediastínica.

Posteriorment, es va demostrar en estudis preliminars, l'ús de l'ultrasonografia endobronquial amb sonda convexa per realitzar PATB en temps real sota la guia directa dels ultrasons[9]. El 2006 es va publicar el primer intent de guia de realització de USEB-PATB[10], actualitzada anys més tard per Tournoy et al[11]. A partir d'aquí, han aparegut múltiples estudis que demostren la utilitat de la USEB-PATB en l'estudi del mediastí i en especial en l'estadiatge ganglionar mediastínic del càncer de pulmó (CP)[10,12,13]. Des de llavors, el paper d'aquesta tècnica s'ha anat incrementant i ha canviat la pràctica habitual en les unitats de pneumologia intervencionista, no només en el diagnòstic i estadiatge del CP, sinó també en el diagnòstic i estadiatge d'altres malalties neoplàsiques toràciques i extra-toràciques així com de malalties pulmonars no tumorals. En la última proposta del mapa ganglionar mediastínic de la setena edició de la classificació TNM (tumor, gangli, metàstasi) del càncer de pulmó, es descriuen les estacions mediastíniques i els seus límits, que serviran de guia per a la USEB[14]. Amb la USEB-PATB es poden estudiar lesions centrals i ganglis mediastínics en les estacions ganglionars paratraqueals (2R, 2L, 3, 4R i 4L), ganglis limfàtics subcarinals (nivell 7) i ganglis limfàtics hiliars (10, 11, i 12)[15].

Si es combina amb la Ultrasonografia Endoscòpica amb ecobroncoscopi (USEB-B) amb punció aspiració (USEB-B-PA), s'augmenta el rendiment diagnòstic vers la via endobronquial[16], però sobretot millora l'abordatge d'algunes estacions, com la 4L i permet abordar estacions paraesofàgiques (8 i 9)[17] i l'estudi de la glàndula suprarenal esquerre

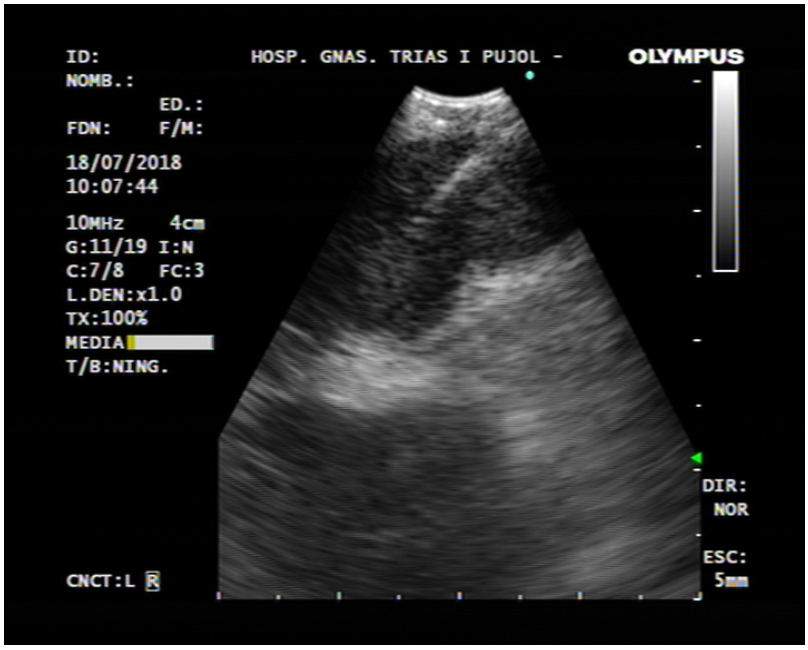

Figura 8. USEB-PATB: Punció de gangli mediastínic.

Encara que la majoria dels estudis inicials es van centrar en l'estadiatge de càncer de pulmó, ja s'havia descrit la USEB-PATB com una està tècnica útil en altres patologies[18–22]. A més, ha demostrat ser una tècnica molt segura, el que li ofereix un gran avantatge respecte la mediastinoscòpia[23]. Les indicacions actuals de la USEB-PATB son: Estadiatge del càncer de pulmó, estudi d'adenopaties mediastíniques en pacients amb malaltia maligna diagnòstic de malalties limfoproliferatives i infeccioses, mostreig de teixits de tumors pulmonars, estudi de lesions mediastíniques i presa de mostres per a estudis de biomarcadors[15,24,25]. Diferents estudis i metaanàlisis han demostrat alta sensibilitat, especificitat i seguretat de l'USEB-TBNA[23,26–28]. L'estudi de la mostra citològica de forma directa per l'anatomopatòleg durant la realització de la USEB-PATB, anomenat "rapid on-site evaluation" (ROSE) ha permès millorar la presa de mostres, i reduir el nombre de puncions i exploracions[29,30].

2. Aplicacions de la USEB-PATB en l'estudi del mediastí.

Encara que la indicació prínceps de la USEB-PATB és el diagnòstic i estadiatge del CP, ha demostrat la seva utilitat en moltes altres aplicacions, com hem vist, que es descriuran a continuació.

2.1. La USEB-PATB en l'estudi de malaltia benigna.

El fet que la USEB-PATB permet avaluar en temps real el ganglis mediastínics i prendre mostres vàlides per a diferents estudis, ha permès l'estudi de malalties benignes que produeixen afectació mediastínica.
Es disposa de bona evidència científica que posa de manifest la utilitat de la USEB-PATB per a l'estudi de la Sarcoïdosi,[20,22,31,32]. S'ha analitzat el paper de la USEB-PATB en el diagnòstic de la Tuberculosi (TB) quan es presenta amb adenopaties mediastíniques, demostrant bons resultats combinant l'estudi citològic amb l'estudi microbiològic i/o molecular de les mostres obtingudes[33,34]. També ha estat descrit el seu paper en el drenatge de lesions quístiques mediastíniques o pleuro-pericardíaques[35,36] o la possibilitat de la valoració de patologia vascular pulmonar[37,38].

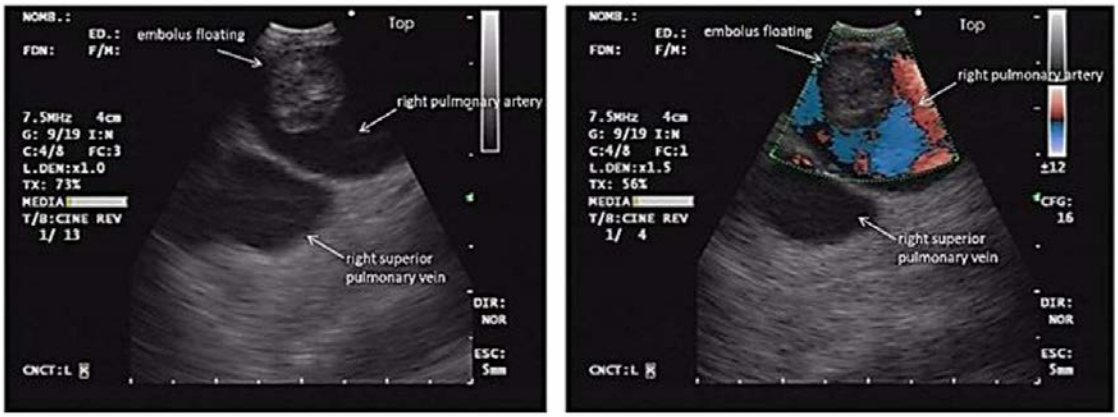

Figura 9. Imatge d'USEB-PATB mostrant un defecte de repleció a l'artèria pulmonar dreta.

2.2. La USEB-PATB pel diagnòstic de tumors centrals.

Molts pacients presenten tumors centrals o masses (paratraqueals o para-esofàgiques) sense lesió endobronquial, no accessibles mitjançant la broncoscòpia flexible estàndard. La USEB-PATB ha demostrat ser un procediment mínimament invasiu i segur, i tenir una alta sensibilitat i especificitat per diagnòstic d'aquests lesions[39,40], evitant altres procediments invasius[41].

2.3. La USEB-PATB pel diagnòstic de limfoma.

Es disposa de molts estudis que han estudiat la utilitat de la USEB-PATB en el diagnòstic de limfoma Tot i que son heterogenis i les xifres de sensibilitat reportades són variables, en molts casos eviten proves més invasives[42], sobretot si es valora la mostra citològica i s'afegeixen tècniques de citometria de flux[43]. Pot ser una bona eina inicial en el diagnòstic o sospita de recidiva de limfomes[44].

2.4. La USEB-PATB en el diagnòstic i estadiatge del CPNCP.

L'estadiatge ganglionar mediastínic és un dels passos transcendentals en el procés d'avaluació dels pacients amb CPNCP. Les tècniques no invasives (PET/TC, TC) son el primer pas per l'estudi del mediastí l'estadiatge, però sovint, els resultats necessiten confirmació histològica, on l'USEB-PATB ha emergit com un procediment mínimament invasiu i molt precís en l'estadiatge ganglionar mediastínic[45].

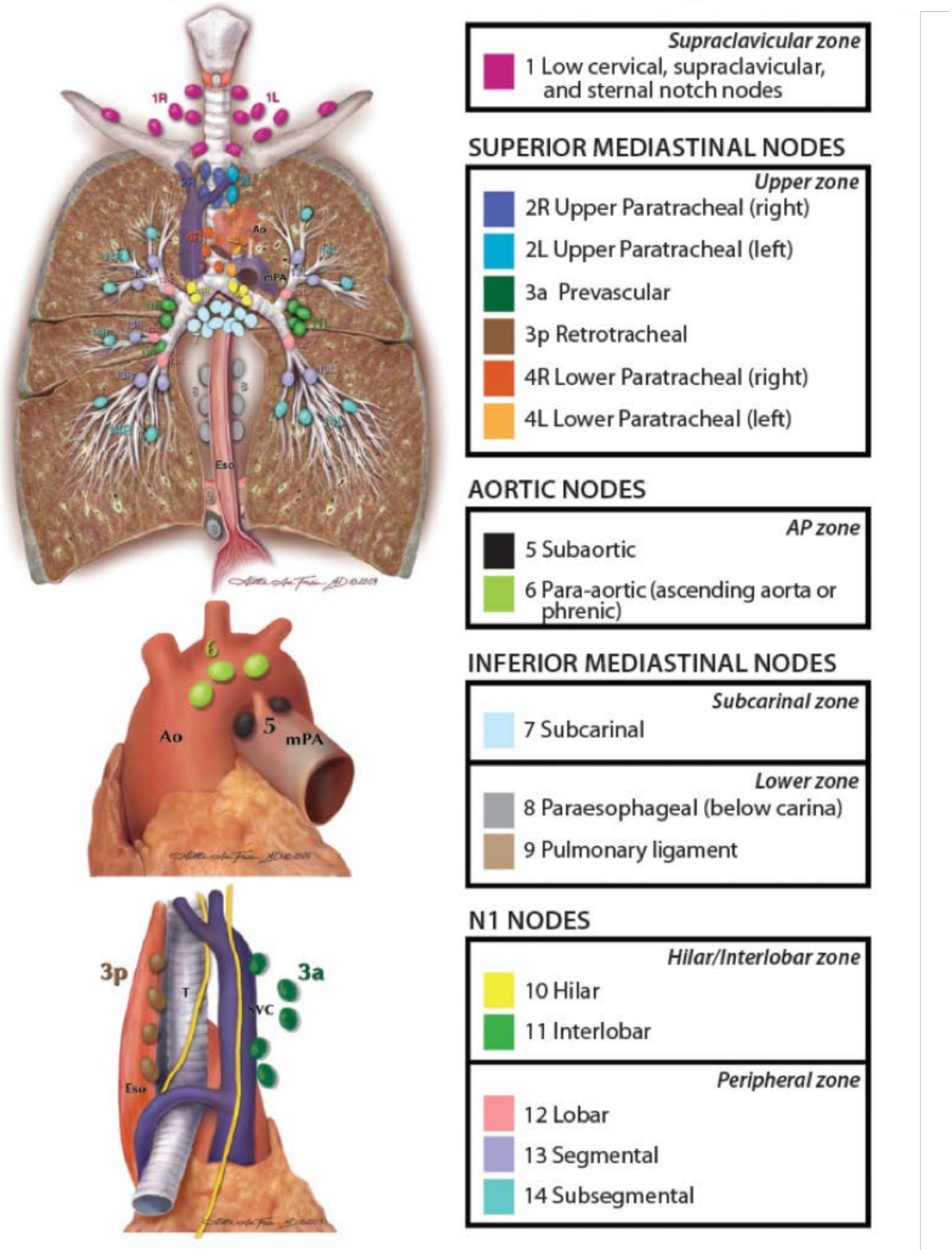

Figura 10. Mapa ganglionar mediastínic. Adaptat de Staging Manual in Thoracic Oncology, 2nd Edition by IASLC.

#1 (Left/Right) Low cervical, supraclavicular and sternal notch nodes

Upper border: lower margin of cricoid cartilage
Lower border: clavicles bilaterally and, in the midline, the upper border of the manubrium, 1R designates right-sided nodes, 1L, left-sided nodes in this region.
#L1 and #R1 limited by the midline of the trachea.

#2 (Left/Right) Upper paratracheal nodes

2R: Upper border: apex of the right lung and pleural space and, in the midline, the upper border of the manubrium
Lower border: intersection of caudal margin of innominate vein with the trachea
2L: Upper border: apex of the left lung and pleural space and, in the midline, the upper border of the manubrium
Lower border: superior border of the aortic arch
As for #4, in #2 the oncologic midline is along the left lateral border of the trachea.

#3 Pre-vascular and retrotracheal nodes

3a: Prevascular - On the right
upper border: apex of chest
lower border: level of carina
anterior border: posterior aspect of sternum
posterior border: anterior border of superior vena cava
3a: Prevascular - On the left
upper border: apex of chest
lower border: level of carina
anterior border: posterior aspect of sternum
posterior border: left carotid artery
3p: Retrotracheal
upper border: apex of chest
lower border: carina

#4 (Left/Right) Lower paratracheal nodes

4R: includes right paratracheal nodes, and pretracheal nodes extending to the left lateral border of trachea
upper border: intersection of caudal margin of innominate vein with the trachea
lower border: lower border of azygos vein
4L: includes nodes to the left of the left lateral border of the trachea, medial to the ligamentum arteriosum
upper border: upper margin of the aortic arch
lower border: upper rim of the left main pulmonary artery

Rusch VW, Asamura H, Watanabe H, Giroux DJ, Rami-Porta R, Goldstraw P. The IASLC lung cancer staging project. A proposal for a new international lymph node map in the forthcoming seventh edition of the TNM classification for lung cancer. J Thorac Oncol 2009; 4: 568-577.

#5 Subaortic (aorto-pulmonary window)

Subaortic lymph nodes lateral to the ligamentum arteriosum
upper border: the lower border of the aortic arch
lower border: upper rim of the left main pulmonary artery

#6 Para-aortic nodes ascending aorta or phrenic

Lymph nodes anterior and lateral to the ascending aorta and aortic arch
upper border: a line tangential to upper border of aortic arch
lower border: the lower border of the aortic arch

#7 Subcarinal nodes

upper border: the carina of the trachea
lower border: the upper border of the lower lobe bronchus on the left; the lower border of the bronchus intermedius on right

#8 (Left/Right) Para-esophageal nodes (below carina)

Nodes lying adjacent to the wall of the esophagus and to the right or left of the midline, excluding subcarinal nodes
upper border: the upper border of the lower lobe bronchus on the left; the lower border of the bronchus intermedius on right
lower border: the diaphragm

#9 (Left/Right) Pulmonary ligament nodes

Nodes lying within the pulmonary ligament
upper border: the inferior pulmonary vein
lower border: the diaphragm

#10 (Left/Right) Hilar nodes

Includes nodes immediately adjacent to the mainstem bronchus and hilar vessels including the proximal portions of the pulmonary veins and main pulmonary artery
upper border: the lower rim of the azygos vein on the right; upper rim of the pulmonary artery on the left
lower border: interlobar region bilaterally

#11 Interlobar nodes

Between the origin of the lobar bronchi
***#11s:** between the upper lobe bronchus and bronchus intermedius on the right
***#11i:** between the middle and lower lobe bronchi on the right

#12 Lobar nodes

Adjacent to the lobar bronchi

#13 Segmental nodes

Adjacent to the segmental bronchi

#14 Sub-segmental nodes

Adjacent to the subsegmental bronchi

Figura 11. Descripció dels límits anatòmics del mapa ganglionar mediastínic. Adaptat de Staging Manual in Thoracic Oncology, 2nd Edition by IASLC.

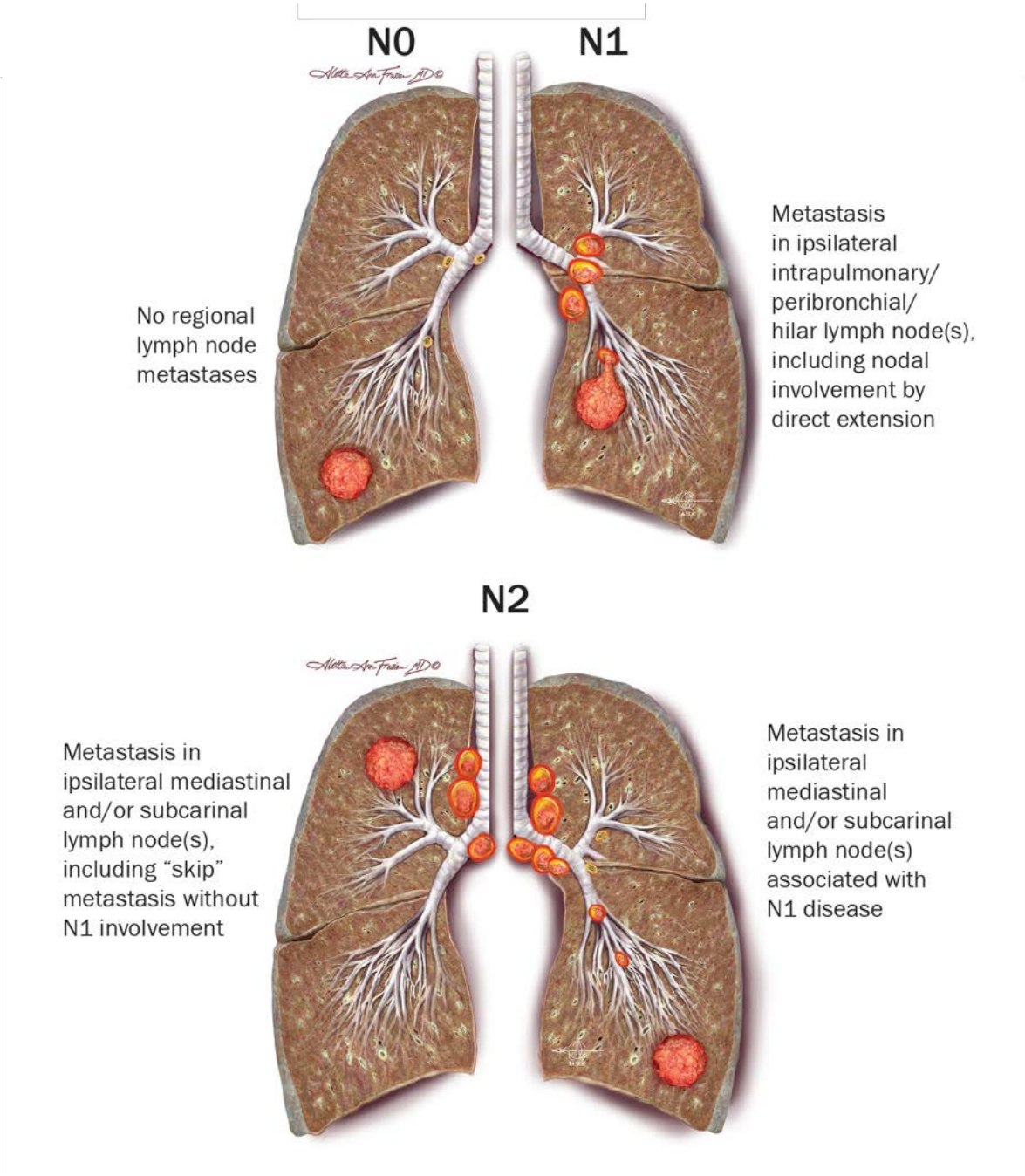

Figura 12. Descripció de l'estadiatge ganglionar del TNM. Adaptat de Staging Manual in Thoracic Oncology, 2nd Edition by IASLC.

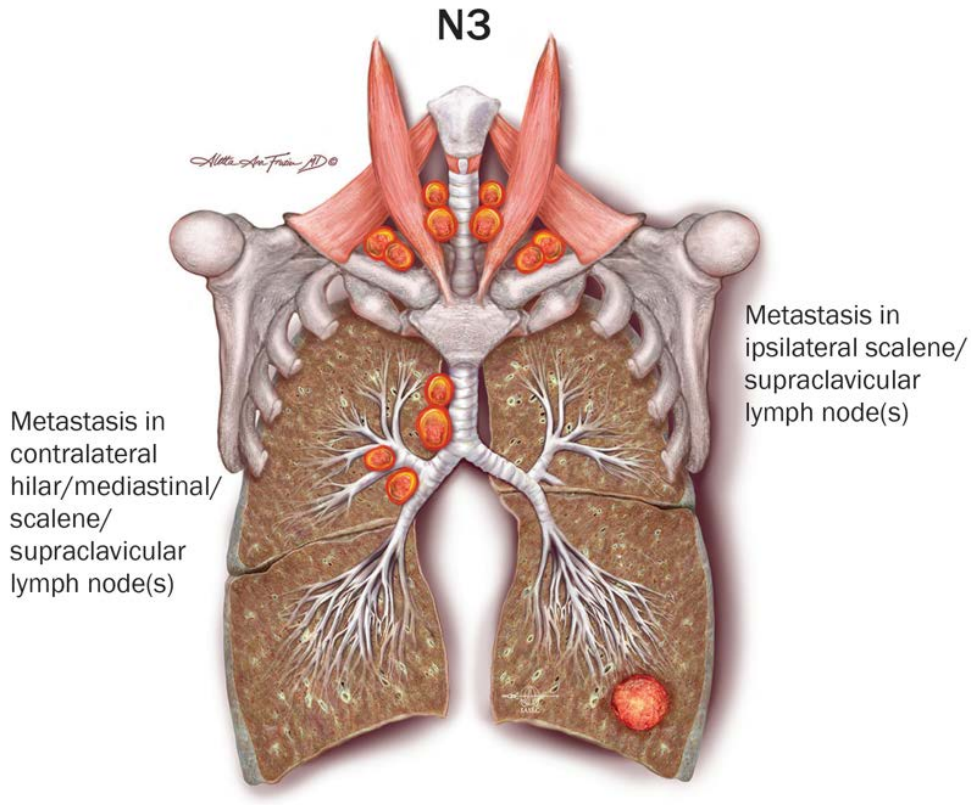

Figura 13. Descripció de l'estadiatge ganglionar del TNM. Adaptat de Staging Manual in Thoracic Oncology, 2nd Edition by IASLC.

N – Regional Lymph Nodes

NX		Regional lymph nodes cannot be assessed
N0		No regional lymph node metastasis
N1		Metastasis in ipsilateral peribronchial and/or ipsilateral hilar lymph nodes and intrapulmonary nodes, including involvement by direct extension
N2		Metastasis in ipsilateral mediastinal and/or subcarinal lymph node(s)
N3		Metastasis in contralateral mediastinal, contralateral hilar, ipsilateral or contralateral scalene or supraclavicular lymph node(s)

Figura 14. Descripció de l'estadiatge ganglionar del TNM. Adaptat de Staging Manual in Thoracic Oncology, 2nd Edition by IASLC.

S'han publicat múltiples estudis i revisions, on es mostra un alt rendiment diagnòstic en l'estadiatge ganglionar mediastínic del CPNCP amb sensibilitats del 88-93% i especificitat del 100%[26,46]. De manera que les guies clíniques sobre el diagnòstic i estadiatge del CPNCP han inclòs la USEB-PTB com una eina clau en l'estadiatge no invasiu del CPNCP, afirmant que la USEB-PATB (amb o sense l'addició del USE-B-PA) es considera com la primera tècnica a utilitzar en l'estudi de l'afectació mediastínica del CPNCP[45,47,48], inclús reemplaçant a les tècniques quirúrgiques[48]. Per això, es recomana la realització de la USEB-PATB de forma sistemàtica, ampliant els requisits de la European Society of Thoracic Surgeons (ESTS) de puncionar com a mínim els ganglis majors de >5mm i els ganglis positius al PET/TC en les estacions 4L, 4R i 7, i valorar les estacions hiliars segons indicació, a puncionar tots els ganglis limfàtics visibles majors de 5mm, amb un mínim de 3 estacions N2-N3, començant per l'estudi de les estacions contralaterals N3, seguit de les estacions N2 i finalment de les N1 si és necessari[49].

Quan els resultats son negatius, sempre s'ha recomanat la realització de mediastinoscòpia (a excepció del mediastí no patològic al PET-TC i centres molt especialitzats)[48,50], tot i que recents estudis pretenen canviar aquesta dinàmica, valorant la possibilitat d'ometre la mediastinoscòpia per millorar en termes de supervivència[51]. De fet, la comparació entre la USEB-PATB i la mediastinoscòpia, mostra resultats similars de rendiment diagnòstic, sensibilitat i valor predictiu negatiu (VPN), amb menys temps d'espera i complicacions[52–54].

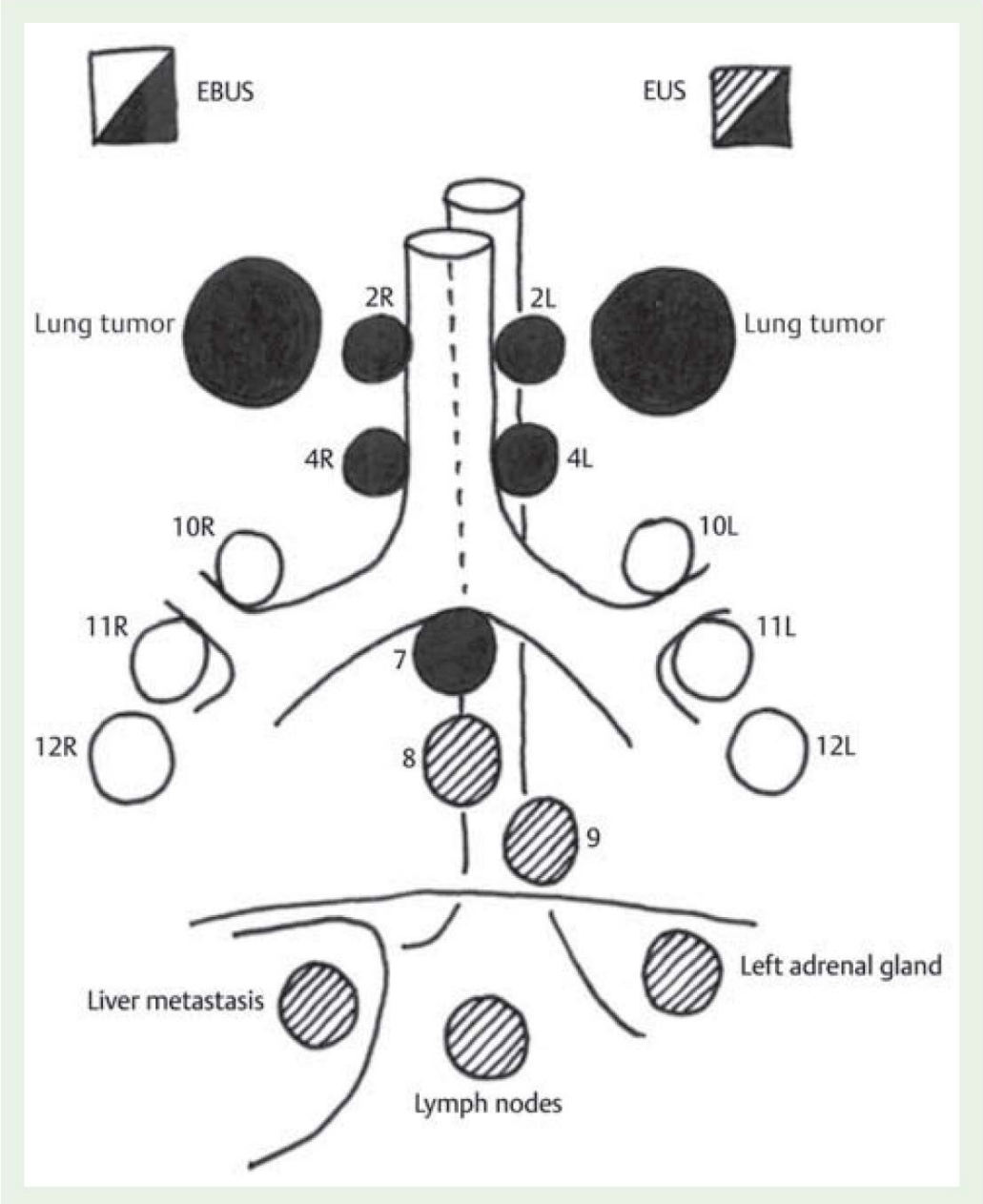

Figura 15. Imatge de l'abordatge ganglionar de USEB-PATB + EUS.

La combinació de la USEB-PATB i USE o USE-B, augmenta les estacions abordables endoscòpicament i alguns estudis han demostrat que els resultats en comparació amb la mediastinoscòpia són favorables a l'estadiatge endoscòpic, millorant la sensibilitat, requerint menys toracotomies[55] i amb menys complicacions[51].

2.5. La USEB-PATB en l'estudi de biomarcadors.

Les mostres obtingudes mitjançant l'USEB-TBNA, han demostrat ser vàlides per a la realització d'estudi moleculars (dianes terapèutiques a diferents malalties neoplàsiques) de diferents tipus. Especialment, la tècnica d'obtenció del bloc cel·lular permet obtenir mostres de qualitat equiparable a les mostres histològiques per l'abordatge d'aquests biomarcadors[56] que són útils tant com a dianes terapèutiques (Figura 16) o com a factors pronòstics[57].

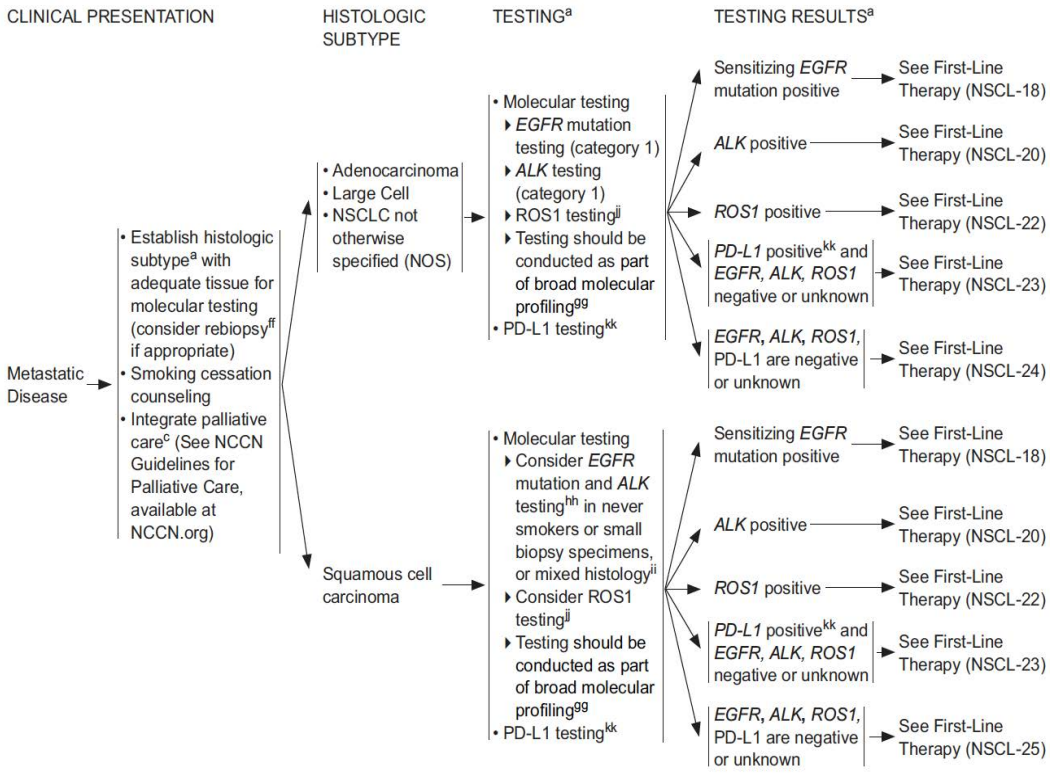

Figura 16. Recomanacions de determinació de dianes terapèutiques en pacients amb CPNCP segons recomanacions de la NCCN (Journal of the National Comprehensive Cancer Network).

Ja des del 2007 es descriuen estudis mitjançant mostres d'USEB-PATB de la mutació del receptor del factor de creixement epidèrmic (EGFR)[58], i posteriorment, múltiples estudis han demostrat que les mostres de la USEB-TBNA permeten estudiar diferents mutacions a més de l'EGFR[24], com els gens KRAS i BRAF i del

gen que codifica la subunitat catalítica de la fosfatidilinositol3-quinasa (PIK3CA)[59,60], el test per la fusió de gens de la quinasa de limfoma anaplàstic (EML4-ALK)[61]. També s'ha descrit la utilitat de la USEB-PATB en l'estudi del "programed Death-ligand 1" (PDL-1)[62] i el proto-oncogen ROS-1[63]. Tanmateix l'USEB-PATB permet la identificació de biomarcadors en fase experimental com ara la determinació de microRNAs com el miR-200c[64] , amplificacions genètiques (MET, FGFR1)[65] i la metilació del ADN[66].

2.6. La USEB-PATB en el re-estadiatge del CPNCP.

Els avenços en el tractament del CPNCP han estat importants en els últims anys, i això comporta de manera progressiva canvis en la resposta i seguiment dels pacients, i per tant, en el procés de re-estadiatge després del tractament d'inducció. De moment disposem de menys evidència científica en aquest camp, respecte el procés d'estadiatge, amb menys sensibilitat i VPN. La combinació de USEB-PATB i USE ha demostrat millorar aquestes xifres[67].

2.7. *La USEB-PATB en la recidiva del CPNCP.*

La USEB-PATB també s'ha mostrat útil en el diagnòstic de recidiva ganglionar de CPNCP prèviament tractats[68,69], tot i que de moment es disposa de poca evidència, especialment en pacients tractats amb cirurgia. Abans de l'USEB-TBNA la mediastinoscòpia era el mètode d'elecció, i tot i que en centres experts, s'ha mostrat útil[70], és difícil i insegura després d'una cirurgia prèvia (re-mediastinoscòpia) i a més els ganglis hiliars no són abordables. Abans de la present tesi, mai s'havia estudiat el paper de l'USE(B) en el diagnòstic de recidiva mediastínica en pacients amb CPNCP sotmesos a cirurgia de resecció pulmonar.

2.8. USEB-PATB en l'estudi de neoplàsies extra-toràciques.

Una gran nombre de tumors extra-pulmonars, fins un 30%, poden produir afectació mediastínica i hiliar, sent els tumors primaris més freqüents els de cap i coll, colorectal, mama, renal, esòfag, gàstric, prostàtic i de melanoma[23,71]. Pocs estudis han demostrat que la USEB-PATB és una prova adequada i amb bona sensibilitat per a l'estudi d'afectació mediastínica de neoplàsies extra-toràciques i que permet obtenir mostres de bona qualitat per a estudis moleculars[65,72,73].

Hipòtesis i Objectius

HIPÒTESIS

1. L'estadiatge mediastínic de forma Sistemàtica (estudi ganglionar des de N3 fins a N1 independentment de les troballes al PET/TC) pot millorar el rendiment diagnòstic de la USEB-PATB en l'estadiatge ganglionar mediastínica respecte l'estadiatge dirigit (punció només dels ganglis patològics al PET/TC).

2. La USEB-PATB pot ser una eina precisa pel diagnòstic de recidives ganglionars mediastíniques en pacients amb càncer de pulmó previ tractat quirúrgicament i la combinació amb la USE(B) n'incrementa el rendiment.

3. Les mostres de metàstasis mediastíniques de càncer de mama obtingudes mitjançant USEB-PATB poden ser útils per a la determinació de dianes terapèutiques.

OBJECTIU PRINCIPAL

1. Avaluar la utilitat a de la Ultrasonografia Endobronquial amb Punció Aspirativa Transtraqueal / Transbronquial en l'estudi del Mediastí patològic en diferents situacions clíniques.

OBJECTIUS

1. Comparar l'estadiatge mediastínic sistemàtic (estudi ganglionar des de N3 fins a N1 independentment de les troballes al PET/TC), mitjançant USEB-PATB, amb l'estadiatge dirigit per PET/TC (punció només dels ganglis patològics al PET/TC), en pacients amb CPNCP i afectació mediastínica.

2. Avaluar la utilitat del USEB-PATB amb combinació de USE(B) en el diagnòstic de recidives ganglionars mediastíniques en pacients amb càncer de pulmó tractat quirúrgicament.

3. Analitzar la idoneïtat de les mostres de USEB-PATB per la identificació de ER, PR i HER2 en pacients amb metàstasi mediastíniques de càncer de mama.

Publicacions

Estudi 1

Systematic compared with targeted staging with endobronquial ultrasound in patients with lung cancer.

José Sanz-Santos, Pere Serra, Mohamed Torky, Felipe Andreo, Carmen Centeno, Leire Mendiluce, Carlos Martínez-Barenys, Pedro López de Castro, Juan Ruiz-Manzano.

Annals of Thoracic Surgery. 2018 Aug; 106(2):398-403.

Factor d'impacte 2017: 3.77

ABSTRACT

***Introducció**: Avaluació de la precisió de l'estadiatge sistemàtic mitjançant ultrasonografia endobronquial amb punció aspiració transbronquial (USEB-PATB) (mostrejant tots els ganglis visibles ≥5mm des de les estacions N3 fins N1 independentment de les característiques del PET-TC) i comparació amb l'estadiatge per USEB-TBNA dirigit (mostrejant només els ganglis positius per PET-TC), en pacients amb CPNCP i estadiatge N2 per PET-TC.*

***Mètodes**: Es tracta d'un estudi retrospectiu de 107 pacients que van ser sotmesos a estadiatge mediastínic sistemàtic per USEB-PATB. Els resultats es van comparar amb l'hipotètic escenari on només els ganglis captans al PET-TC fossin mostrejats.*

***Resultats**: El USEB-PATB sistemàtic va demostrar malaltia N3 en 3 pacients, N2 en 60 pacients (42 estació única o N2a i 18 multi-estació o N2b) i 44 pacients van ser finalment N0/1. Dels 44 pacients estadiats N0/N1 després de l'USEB, 7 van ser sotmesos a mediastinoscòpia que no va mostrat malaltia mediastínica, i en 6 d'aquests 7 pacients es va procedir a resecció quirúrgica, que no va mostrar malaltia mediastínica en cap d'ells. 34 pacients van ser sotmesos directament a resecció quirúrgica després de l'USEB-PATB, on la dissecció ganglionar sistemàtica quirúrgica demostrà malaltia N0/N1 en 30 pacients i malaltia N2 en 4 (un N2b amb PET-TC que mostrava malaltia N2a i 3 N2a). La sensibilitat, especificitat, valor predictiu negatiu, valor predictiu positiu i precisió de l'USEB-PATB sistemàtic va resultar del 94%, 100%, 90%, 100% i 96% respectivament. Comparat amb l'USEB-PATB dirigit, l'USEB-PATB sistemàtica va proporcionar informació clínica rellevant addicional en 14 casos (13%): 3 casos de malaltia N3 que haguessin passat desapercebuts en un procediment EBUS dirigit i 11 casos de N2b que hagessin estat estadiats N2a.*

***Conclusions**: A la pràctica clínica, l'estadiatge sistemàtic del mediastí mitjançant USEB-PATB, independentment dels resultats del PET-TC, aporta més informació que l'estadiatge dirigit. L'estadiatge sistemàtic es molt més recomanable que l'estadiatge dirigit.*

GENERAL THORACIC

Systematic Compared With Targeted Staging With Endobronchial Ultrasound in Patients With Lung Cancer

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Bronchoscopy Unit, Pulmonology Department, Hospital Germans Trias i Pujol, Badalona; Pulmonology Department, Hospital Universitari Mútua Terrassa, University of Barcelona, Terrassa; Medicine Department, Universitat Autònoma de Barcelona, Cerdanyola; and Thoracic Surgery Department, Hospital Germans Trias i Pujol, Badalona, Spain

Background. To evaluate the accuracy of systematic mediastinal staging by endobronchial ultrasound transbronchial needle aspiration (EBUS-TBNA) (sampling of all visible nodes measuring ≥5 mm from stations N3 to N1 regardless of their positron emission tomography/computed tomography [PET/CT] features) and compare this staging approach with targeted EBUS-TBNA staging (sampling only 18F-fluorodeoxyglucose [FDG]-avid nodes) in patients with N2 non-small cell lung cancer on PET/CT.

Methods. Retrospective study of 107 patients who underwent systematic EBUS-TBNA mediastinal staging. The results were compared with those of a hypothetical scenario where only FDG-avid nodes on PET/CT would be sampled.

Results. Systematic EBUS-TBNA sampling demonstrated N3 disease in 3 patients, N2 disease in 60 (42 single-station or N2a, 18 multiple-station or N2b) and N0/N1 disease in 44. Of these 44, 7 underwent mediastinoscopy, which did not show mediastinal disease; 6 of the 7 proceeded to lung resection, which also showed no mediastinal disease. Thirty-four N0/N1 patients after EBUS-TBNA underwent lung resection directly: N0/N1 was found in 30 and N2 in 4 (1 N2b with a PET/CT showing N2a disease, 3 N2a). Sensitivity, specificity, negative predictive value, positive predictive value, and overall accuracy of systematic EBUS-TBNA were 94%, 100%, 90%, 100% and 96%, respectively. Compared with targeted EBUS-TBNA, systematic EBUS-TBNA sampling provided additional important clinical information in 14 cases (13%): 3 N3 cases would have passed unnoticed, and 11 N2b cases would have been staged as N2a.

Conclusions. In clinical practice, systematic sampling of the mediastinum by EBUS-TBNA, regardless of PET/CT features, is to be recommended over targeted sampling.

(Ann Thorac Surg 2018;106:398–403)

Despite advances in early diagnosis, staging and treatment, lung cancer remains the most commonly diagnosed cancer and the leading cause of cancer-related mortality worldwide [1]. In patients with non-small cell lung cancer (NSCLC) without distant metastases, accurate staging of the mediastinal lymph nodes is essential to determine appropriate therapy and to evaluate prognosis. Given that the extension of nodal disease correlates with prognosis [2], the 8th edition of the Tumour, Node and Metastasis (TNM) classification for lung cancer proposed a subclassification of the N categories using new descriptors [2]: N1a, single-station N1 involvement; N1b, multiple-station N1 involvement; N2a1, single-station N2 involvement without N1 disease (skip metastases); N2a2, single-station N2 with N1 disease; N2b, multiple-station N2 involvement; and N3.

Computed tomography (CT) combined with 18F-fluorodeoxyglucose (FDG) positron emission tomography (PET) is routinely used for non-invasive staging of patients with NSCLC. When PET/CT results are positive, however, they require pathological confirmation by invasive procedures [3]. Over the last few years, endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) has emerged as a minimally invasive and highly accurate procedure for staging mediastinal lymph nodes [3]. Moreover, clinical-practice guidelines recommend EBUS-TBNA as the first approach for invasive staging of the mediastinum, replacing surgical procedures [3, 4].

The latest European Society of Thoracic Surgeons (ESTS) guidelines for preoperative mediastinal lymph node staging of NSCLC recommend that EBUS-TBNA should sample, as a minimum requirement, the largest

Accepted for publication Feb 26, 2018.

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0003-4975/$36.00
https://doi.org/10.1016/j.athoracsur.2018.02.088

Ann Thorac Surg
2018;106:398–403

nodes measuring greater than 5 mm on ultrasonography examination in nodal stations 4R, 4L, and 7 as well as FDG-avid nodes within each of these nodal stations—an approach that is similar to quality standards for cervical mediastinoscopy. Despite these recommendations, a recently published survey [5] demonstrated that this staging strategy is not widely followed in many institutions, as targeted EBUS-TBNA staging (sampling exclusively the FDG-avid nodes) is the usual practice. Other authors have proposed extending the minimum ESTS requirements and performing systematic EBUS-TBNA staging of all lymph nodes measuring 5 mm or more, with a minimum of 3 N2/N3 stations, starting with an examination of the contralateral N3 nodal stations, followed by N2 stations, and finally N1 lymph nodes when required [6]. Although a wealth of evidence has shown that EBUS-TBNA has a higher sensitivity and specificity for mediastinal staging compared with PET/CT [7, 8], to the best of our knowledge, no studies have compared systematic EBUS-TBNA versus targeted EBUS-TBNA staging strategies.

We have retrospectively evaluated the accuracy of systematic EBUS-TBNA mediastinal staging (sampling of all visible nodes measuring 5 mm or more from stations N3 to N1 regardless of their PET/CT features) and compared this staging strategy with a hypothetical scenario of targeted EBUS-TBNA staging (sampling of only FDG-avid nodes) in the same series of patients.

Patients and Methods

Design

We conducted a retrospective single-center study including patients diagnosed with NSCLC, with a PET/CT showing FDG-avid N2 nodes, who underwent systematic EBUS-TBNA mediastinal staging. We compared the results of the systematic staging with those of a hypothetical scenario of targeted staging, where only FDG-avid nodes on PET/CT would be sampled.

Patients

Patients with confirmed or suspected NSCLC with a PET/CT showing FDG-avid N2 nodes, but no distant metastases, who underwent systematic EBUS-TBNA for mediastinal staging from January 2014 to December 2016 were included. FDG-avid N2 nodes were defined by an absolute maximum standardized uptake value (SUV max) of 4.5 or greater, and/or a ratio between the SUV max of the mediastinal lymph node and the SUV max of the primary tumor of 0.5 or more [4, 9]. In the geographical area where the study was performed, there is no endemic pulmonary mycosis or pulmonary granulomatous disease. Thus, these conditions do not represent a significant problem in lung cancer staging and all cases with suspicious PET/CT nodes undergo invasive staging.

Every patient with N0/N1 tumours after EBUS-TBNA was assessed independently by a multidisciplinary thoracic tumor team and then underwent mediastinoscopy and/or lung resection. Patients with enlarged nodes and high FDG uptake and those with comorbidities and/or high surgical risk underwent mediastinoscopy to rule out nodal disease. Patients who did not meet these criteria underwent resection without prior mediastinoscopy. Operability was assessed by physical examination, electrocardiogram, and pulmonary function tests. Lung resection with nodal dissection was the standard management of patients who were considered candidates for surgery [10]. Patients with negative systematic EBUS-TBNA who could not undergo resection were excluded from the analysis of negative predictive value (NPV).

The internal review board of our institution approved the study (reference number PI-15-127) and written informed consent was obtained from all participating patients.

Systematic EBUS-TBNA Staging

Systematic EBUS-TBNA staging included sampling of any lymph node measuring 5 mm or more starting from N3 hilar stations, proceeding to mediastinal N3 stations, then N2 stations if the rapid on-site examination (ROSE) by the pathologist did not detect any N3 malignant node. If N3 disease was detected, the procedure was then terminated. In N2 stations, the staging started from non–FDG-avid nodes and proceeded to FDG-avid nodes if the ROSE did not detect any malignant node. In case of N2 disease, the needle was changed to avoid contamination and the rest of the N2 stations were sampled. FDG-avid nodes were considered nonmalignant only after 3 aspirates showing lymphocytes and no malignant cells on the ROSE. If the ROSE did not detect N2 disease, N1 stations were sampled when required. The entire process took from 75 to 120 minutes.

EBUS was performed using a flexible bronchoscope (BFUC180F, Olympus Optical Co Ltd, Tokyo, Japan) with a distal probe capable of producing linear parallel scans of the mediastinal and peribronchial tissues and a working channel suited for the performance of TBNA under direct ultrasound guidance. Local anesthesia and sedation were achieved using topical lidocaine spray and intravenous midazolam, propofol, and/or fentanyl in accordance with standard recommendations [11]. Mediastinal, hilar, and lobar nodes with a short-axis diameter of 5 mm or greater identified during the procedure were targeted under direct ultrasound visualization with a 22-gauge cytology needle specially designed for EBUS-TBNA (NA-201SX-4022, Olympus Optical Co Ltd) [12]. The needle was guided beyond the bronchoscope channel and then pushed forward from the sheath to be inserted into the tracheal or bronchial wall under ultrasound guidance. Once the needle tip was inside the target, negative pressure was maintained by a syringe at the proximal end of the catheter while the needle was pushed back and forth. Then suction was released before removal from the target structure.

Pathologic Examination

The aspirated material in the needle was recovered and the specimens were placed on slides and examined in situ by the pathologist, who classified them as "normal tissue negative for malignancy", "metastatic", or "nonsatisfactory". Nodes classified as nonsatisfactory were

sampled as many times as needed to obtain representative diagnostic material.

Surgical Staging

Mediastinoscopy was performed based on ESTS recommendations and included biopsy of right and left inferior paratracheal (nodal stations 4R and 4L, respectively) and subcarinal (nodal station 7) lymph nodes, regardless of nodal size [4]. Systematic nodal dissection was performed in patients undergoing resection according to the recommendations of The Bronchogenic Carcinoma Cooperative Group of the Spanish Society of Pneumology and Thoracic Surgery [13]. Systematic nodal dissection consisted of the excision of all lymph nodes from the ipsilateral, mediastinal, and subcarinal stations.

Statistical Analysis

Data were entered into a database and analyzed using SPSS software (2009, PASW Statistics for Windows, version 18.0; Chicago, IL, SPSS Inc). Categorical variables were expressed as absolute and relative frequencies, continuous variables as means plus standard deviation (SD), and nonnormally distributed data as medians plus interquartile range. The sensitivity, specificity, NPV, positive predictive value (PPV) and overall accuracy of systematic EBUS-TBNA staging were calculated using the standard formulas. The benefits of systematic EBUS-TBNA staging compared with targeted EBUS-TBNA staging (a hypothetical scenario where only FDG-avid nodes would be sampled) were estimated by measuring (1) the number of patients with N3 disease unnoticed on PET/CT and diagnosed by systematic EBUS-TBNA staging and (2) the number of patients with N2 disease in non–FDG-avid stations diagnosed by systematic EBUS-TBNA staging.

Results

A total of 107 patients were included. Patient characteristics are shown in Table 1. All patients had been staged as N2 on PET/CT: 78 as N2a (single-station N2) and 29 as N2b (multiple-station N2). A mean of 7 (SD ± 3) nodes per patient and a mean of 4 (SD ± 1.1) nodal stations per patient were sampled during the systematic EBUS-TBNA staging procedure.

Figure 1 shows the results of the systematic EBUS-TBNA staging. Systematic sampling identified N3 disease in 3 patients (2.8%), N2 disease in 60 patients (56.1%) (42 with N2a disease and 18 with N2b disease), and N0/N1 disease in 44 patients (41.1%). Systematic staging provided additional important clinical information compared with targeted staging in 14 cases (13%), all of which were upstaged. The three cases staged as N3 by systematic EBUS-TBNA would have remained undetected. In addition, of the 18 patients with tumors staged as N2b by systematic EBUS-TBNA, 11 (61%) presented N2a involvement on PET/CT and therefore would have been staged as N2a in a targeted EBUS-TBNA procedure (Table 2). Figure 2 displays an example of a patient whose tumor was upstaged from N2a to N2b by systematic EBUS-TBNA staging. A hypothetical targeted EBUS-TBNA staging procedure would have confirmed N2a disease, whereas the systematic staging procedure, which sampled more nodes, identified N2b disease.

Table 1. Characteristics of the 107 Patients Included in This Study

Characteristics	Values
Male sex, n (%)	91 (85)
Age, years, m ± SD	66 ± 8.1
Histological diagnosis, n (%)	
Adenocarcinoma	36 (33.7)
Squamous cell carcinoma	47 (43.9)
Large cell carcinoma	1 (0.9)
Non-small cell lung cancer not otherwise specified	23 (21.5)
Tumor location, n (%)	
Right upper lobe	47 (43.9)
Middle lobe	5 (4.7)
Right lower lobe	24 (22.4)
Left upper lobe	23 (21.5)
Left lower lobe	8 (7.5)
SUV max of N2 nodes, mean (interquartile range)	6.25 (3.3-10.5)

All patients had tumours staged as N2 on PET-CT.

SUV max = maximum standardized uptake value.

Of the 44 patients staged N0/N1 after EBUS-TBNA, 7 underwent cervical mediastinoscopy, 34 proceeded to surgery directly, and 3 had neither mediastinoscopy nor surgery (Fig 1). Mediastinal disease was not detected in any of the 7 patients undergoing mediastinoscopy, 6 of whom then proceeded to lung resection with systematic nodal dissection, where again no mediastinal nodal involvement was observed. The seventh patient died from a stroke and was excluded from the NPV analysis. Among the 34 patients who underwent lung resection directly, systematic nodal dissection revealed N0/N1 disease in 30 and N2 disease in the remaining 4. Of these 4 patients with a false-negative EBUS-TBNA, 3 had N2a disease and 1 had N2b disease, although PET/CT had shown N2a disease. Among the 3 patients who did not undergo either mediastinoscopy or lung resection, 2 had brain metastases detected by brain magnetic nuclear resonance after a negative PET/CT, and the third died from an intestinal perforation. These 3 cases were excluded from the NPV analysis.

Two patients (1.8%) presented complications related to EBUS-TBNA. One patient had a pneumothorax that required chest tube drainage. Another presented a mediastinal hematoma [14] that was attributed to accidental puncture of an ectopic bronchial artery and required hospital admission and observation for 72 hours. EBUS-TBNA was completed 1 week later.

Sensitivity, specificity, NPV, PPV and overall accuracy of systematic EBUS-TBNA for the diagnosis of mediastinal metastasis was 94%, 100%, 90%, 100%, and 96.1%, respectively. Three patients (2.8%) with undetected N3 disease on PET/CT were diagnosed by systematic EBUS-TBNA staging. The number of systematic EBUS-

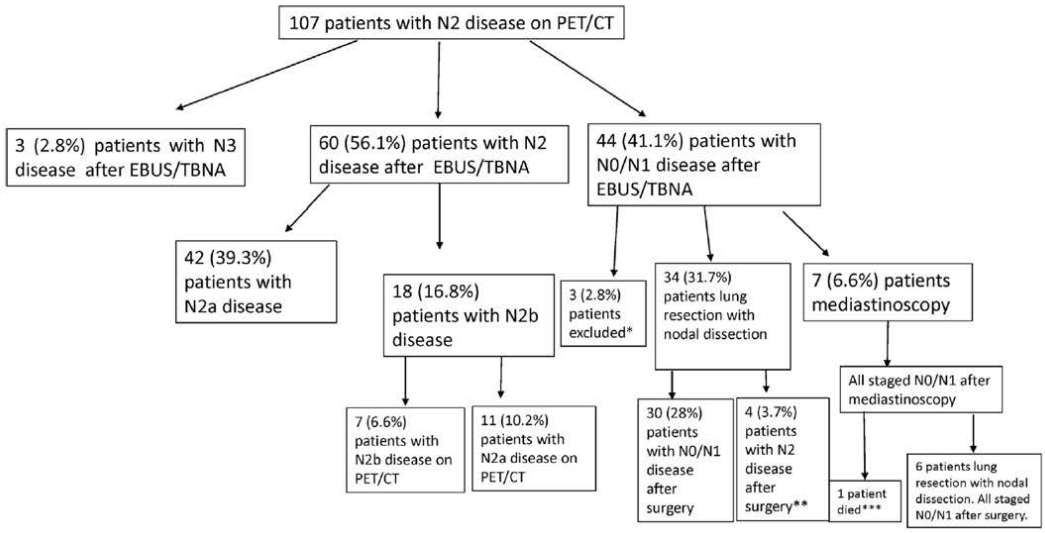

Fig 1. Flow chart showing the results of EBUS-TBNA staging and subsequent assessments. (EBUS-TBNA = endobronchial ultrasound transbronchial needle aspiration; PET/CT = positron emission tomography/computed tomography.)

TBNA procedures needed to diagnose an N3 case undetected on PET/CT was 35.6. Eleven patients (10.2%) staged as N2a by PET/CT were diagnosed with N2b disease by systematic EBUS-TBNA staging. The number of systematic EBUS-TBNA procedures needed to diagnose an N2b disease case undetected on PET/CT was 9.36.

Comment

We have examined the accuracy of a systematic EBUS-TBNA staging procedure that sampled each mediastinal node measuring 5 mm or more regardless of PET/CT features. Our findings indicate that compared with targeted sampling of only FDG-avid nodes, systematic sampling can provide important additional clinical information in patients with suspected N2 disease on PET/CT. Moreover, our results support the principle that thoroughness is the best basis for proper mediastinal staging in patients with NSCLC [15].

Although there is agreement that negative results of EBUS-TBNA can be unreliable if minimum requirements are not met during the procedure, positive results are accepted without concern. Indeed, false-positive results are exceptional [16, 17] but, even with a positive result, EBUS-TBNA mediastinal staging can be suboptimal if only FDG-avid nodes are explored. The revised ESTS guidelines for preoperative mediastinal lymph node staging for NSCLC recommend that the minimum requirement for an invasive staging procedure should be sampling only the largest node of stations 4R, 4L, and 7 and every FDG-avid node within these stations [4]. These minimum requirements apply to both EBUS-TBNA and to mediastinoscopy. To our mind, however, perhaps the goal should be for EBUS-TBNA to sample all nodes, thus making it more consistent with surgical nodal dissection, which removes all visible nodes. In our series, the mean number of sampled nodes on EBUS-TBNA per patient was 7 (SD ± 3) overall, and 8.3 (SD ± 2.9) in the group of patients staged N0/N1 by systematic EBUS-TBNA. Furthermore, the number of aspirates was even higher, as FDG-avid nodes were sampled 3 times before ruling out malignancy and aspirates with nonsatisfactory specimens had to be repeated in order to be considered

Table 2. Comparison of Staging According to PET/CT, Targeted EBUS-TBNA, and Systematic EBUS-TBNA

PET/CT Staging (n)	Targeted EBUS-TBNA (n)	Systematic EBUS-TBNA (n)
N2b (29)	N2b (7)	N2b (7)
	N2a (9)	N2a (9)
	N0/1 (13)	N0/1 (13)
N2a (78)	N2a (47)	N3 (3)
		N2b (11)
		N2a (33)
	N0/1 (31)	N0/1 (31)

The targeted EBUS-TBNA column expresses the results of sampling only the FDG-avid nodes on PET/CT. Tumors staged N2a or N2b by PET/CT might have been downstaged or maintained their stage after targeted EBUS-TBNA but would never have been upstaged because non–FDG-avid nodes would have not been sampled. Fourteen (13%) patients with N2a tumors by PET/CT and targeted EBUS-TBNA were upstaged after systematic EBUS-TBNA (3 to N3 and 11 to N2b).

EBUS-TBNA = endobronchial ultrasound transbronchial needle aspiration; PET/CT = positron emission tomography/computed tomography.

Ann Thorac Surg
2018;106:398–403

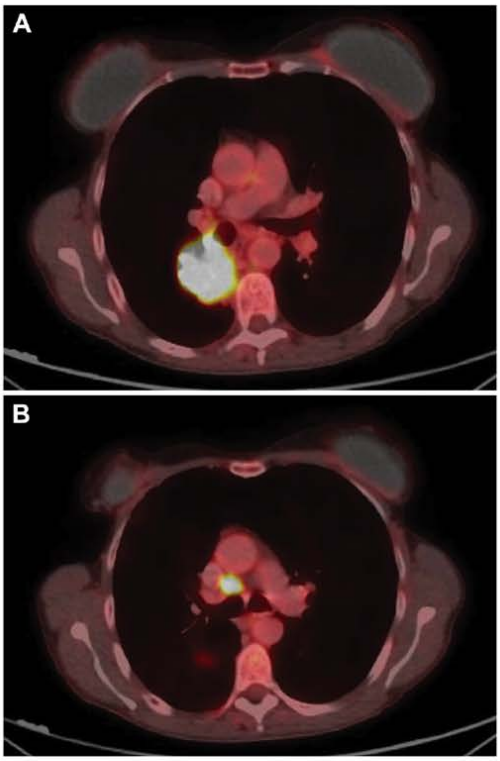

Fig 2. A 59-year-old woman with a pulmonary mass in the right lower lobe presented with a PET/CT showing high FDG uptake (A) in the pulmonary mass and (B) in a right lower paratracheal (4R) node with no other mediastinal uptake. The systematic EBUS-TBNA staging started sampling the N3 nodes: a left hilar (11L) node and two left lower paratracheal (4L) nodes. The ROSE did not show malignancy in any of them. The staging procedure then proceeded to the subcarinal nodal station, where a node was consistent with malignacy. Once the needle was changed to avoid contamination, the 4R node was sampled and the ROSE confirmed multiple nodal disease (N2b). In a targeted EBUS-TBNA staging scenario, only one node (4R) would have been sampled. Although the length of the procedure would have been shorter with targeted EBUS-TBNA sampling of only 1 node, this would have notably reduced the clinical information compared with that obtained with systematic EBUS-TBNA, where 5 nodes were sampled.

diagnostic. Obviously, sampling more nodes increases the accuracy of EBUS-TBNA staging and we recommend that EBUS-TBNA staging should include sampling every mediastinal node measuring 5 mm or greater and not only 1 node per mediastinal nodal station. Our study demonstrates that in a population with N2 disease on PET/CT, the probability of occult mediastinal metastases is high and sampling limited to the FDG-avid nodes can lead to loss of relevant clinical information in almost 15% of cases. The role of PET/CT in lung cancer staging is unquestionable, but once a patient is selected for invasive staging of the mediastinum, this should not be based only on PET/CT features. Although systematic EBUS-TBNA is a labor-intensive and time-consuming procedure that can take between 75 and 120 minutes, and may require a longer anesthesia time than that required for targeted EBUS-TBNA, our results show that it is worth performing.

There is no consensus regarding the best treatment for N2b disease. Nonetheless, it is well known that the extent of nodal disease has prognostic impact, with lower rates of survival in patients with N2b disease compared with those with N2a disease [2]. In our series of patients with N2 disease identified on PET/CT, almost one third were staged with N2b disease by systematic EBUS-TBNA, although two thirds had a single-station involvement on PET/CT and the N2b disease would have been missed by targeted EBUS-TBNA. In lung cancer, the N component of the TNM classification is based on the location of the nodes alone regardless of the number (nN) and/or the size of metastatic nodes. Some authors have demonstrated that nN is a better prognostic determinant than the location-based pN classification [18]. This is probably because the location-based classification cannot reflect the tumor burden at metastatic lymph nodes. Nevertheless, nN is not easily assessed in clinical practice, except by pathologic staging in patients who have undergone nodal dissection at the time of tumor resection. Currently available tests for clinical staging do not permit counting malignant nodes except with transcervical lymphadenectomies—video-assisted mediastinal lymphadenectomy [19] and trans-cervical extended mediastinal lymphadenectomy [20]. In this setting, EBUS-TBNA has an advantage over image-based techniques in differentiating nodes separately. As our study demonstrates, EBUS-TBNA can distinguish between single-station and multiple-station nodal disease.

Another advantage of systematic EBUS-TBNA staging is related to the paradigm shift in radiotherapy techniques for patients with NSCLC. Traditionally, NSCLC patients have been treated by means of elective nodal irradiation, a technique based on targeting the primary tumor as well as the ipsilateral hilar and mediastinal lymph nodes, even if there is no evidence of clinical involvement of these nodal stations. More recently, in many institutions, NSCLC patients are treated with another technique, called involved field radiotherapy (IFRT) [21]. IFRT includes only the primary tumor and clinically involved nodal stations, which allows a higher radiation dose to the primary tumor and a lower toxicity risk, with the same incidence of nodal failure as elective nodal irradiation. PET/CT is the current standard for dose planning in the radiotherapy workup of patients with NSCLC [22]. According to our results, 21% of patients with NSCLC eligible for IFRT would be undertreated, if the dose planning were based only on results of PET/CT and targeted EBUS-TBNA staging.

Proceeding directly to surgery after a negative systematic EBUS-TBNA without a mediastinoscopy can be a controversial approach. In our series, only 7 of 44 patients with N0/N1 tumours identified by EBUS-TBNA

underwent mediastinoscopy. Mediastinal metastases were not detected in any of the 7. Six then proceeded to lung resection, which also showed no mediastinal disease in the resected specimens. These findings indicate that even in a population with a high prevalence of N2 disease, systematic EBUS-TBNA can have a high NPV. Our results, however, have to be considered in a setting of an experienced endosonography institution with previously reported high NPV for lung cancer staging [23]. Adding mediastinoscopy to a negative EBUS-TBNA has been shown to increase the sensitivity for detection of nodal metastases [24], is recommended by current staging guidelines, and is yet to be considered the standard of care.

Our study has two main limitations: its retrospective nature and the consequent post hoc comparison with the hypothetical targeted EBUS-TBNA staging. Despite these limitations, the higher reliability of systematic sampling over targeted sampling is evident and clinically important.

In conclusion, our study demonstrates that systematic EBUS-TBNA sampling of the mediastinum, regardless of PET/CT features, provides additional important clinical information compared with targeted sampling of only FDG-avid nodes. We recommend the use of systematic EBUS-TBNA staging in routine clinical practice.

The authors wish to thank Dr Ramón Rami-Porta, from the Thoracic Surgery Service of Hospital Universitari Mútua Terrassa, for revising the manuscript.

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Estudi 2

Transbronquial and transesophageal fine-needle aspiration using a single ultrasound bronchoscope in the diagnosis of locoregional recurrence of surgically-treated lung cancer.

José Sanz-Santos, Pere Serra, Felipe Andreo, Mohamed Torky, Carmen Centeno, Teresa Morán, Enric Carcereny, Esther Fernández, Samuel García-Reina and Juan Ruiz-Manzano.

BMC Pulmonary Medicine (2017) 17:46.

Factor d'impacte a 2017: 2.721.

ABSTRACT

***Introducció**: El present estudi pretén avaluar la utilitat de l'USEB-PATB en el diagnòstic de la recidiva local de càncer de pulmó en una cohort de pacients prèviament tractats quirúrgicament i descriure l'experiència inicial del USE-PA en aquest escenari.*

***Mètodes**: Es van estudiar retrospectivament les dades clíniques de tots els pacients amb càncer de pulmó tractat quirúrgicament que van ser remesos a la unitat de broncoscòpies per sospita de recidiva local. Es va estudiar la sensibilitat, especificitat, valor predictiu negatiu, valor predictiu positiu i precisió de l'USEB-PATB pel diagnòstic de recidiva local.*

***Resultats**: Es van incloure 73 pacients. L' USEB-PATB va confirmar malignitat en 40 pacients: 34 amb recidiva local confirmada i 6 van presentar un tumor metacrònic. Dels 33 pacients amb USEB-PATB sense diagnòstic de malignitat, 2 van resultar malalties benignes específiques, 26 van prosseguir amb seguiment radiològic i 5 van ser sotmesos a cirurgia. Dels 26 pacients en seguiment radiològic, 18 es van mantenir estables en el temps, 3 van presentar progressió radiològica i 5 progressió extra-toràcica. Dels 5 pacients sotmesos a cirurgia, 3 van presentar tumors metacrònic, un va mostrar invasió ganglionar i un va resultar negatiu. 7 pacients van ser sotmesos a USE(B)-PA, dels quals en 4 es va confirmar la recidiva. La sensibilitat, especificitat, valor predictiu negatiu, valor predictiu positiu i precisió diagnòstica de l'USEB-PATB pel diagnòstic de recidiva local va ser del 80.9%, 100%, 69.2%, 100% i 86.6% respectivament.*

***Conclusions**: L'USEB-PATB és un procediment precís pel diagnòstic de la recidiva local de pacients amb càncer de pulmó tractat quirúrgicament. L'USE-PA combinat amb l'USEB-PATB amplia el rendiment diagnòstic respecte l'USEB-PATB sol.*

Sanz-Santos *et al. BMC Pulmonary Medicine* (2017) 17:46
DOI 10.1186/s12890-017-0388-4

BMC Pulmonary Medicine

RESEARCH ARTICLE Open Access

Transbronchial and transesophageal fine-needle aspiration using a single ultrasound bronchoscope in the diagnosis of locoregional recurrence of surgically-treated lung cancer

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Abstract

Background: The present study sought to evaluate the usefulness of EBUS-TBNA in the diagnosis of locoregional recurrence of lung cancer in a cohort of lung cancer patients who were previously treated surgically, and describe our initial experience of EUS-B-FNA in this clinical scenario.

Methods: We retrospectively studied the clinical records of all patients with a previous surgically-treated lung cancer who were referred to our bronchoscopy unit after suspicion of locoregional recurrence. The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy of EBUS-TBNA for the diagnosis of locoregional recurrence were evaluated.

Results: Seventy-three patients were included. EBUS-TBNA confirmed malignancy in 40 patients: 34 confirmed to have locoregional recurrence, six had metachronous tumours. Of the 33 patients with non-malignant EBUS-TBNA; 2 had specific non-malignant diseases, 26 underwent radiological follow up and 5 patients underwent surgery. Of the 26 patients who had radiological follow up; 18 remained stable, three presented thoracic radiological progression and 5 presented extrathoracic progression. Of the 5 patients who underwent surgery; 3 had metachronous tumours, one confirmed to be a true negative and one presented nodal invasion. Seven patients underwent EUS-B-FNA, four of them confirmed to have recurrence. The sensitivity, specificity, NPV, PPV and overall accuracy of EBUS-TBNA for the diagnosis of locoregional recurrence were 80.9, 100, 69.2, 100 and 86.6% respectively.

Conclusions: EBUS-TBNA is an accurate procedure for the diagnosis of locoregional recurrence of surgically-treated lung cancer. EUS-B-FNA combined with EBUS-TBNA broads the diagnostic yield of EBUS-TBNA alone.

Keywords: Lung cancer, Recurrence, Surgically-treated, Endobronchial Ultrasound, Transbronchial needle aspiration, Endoscopic ultrasound, Fine needle aspiration

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Background

Lung cancer is the leading cause of cancer-related mortality worldwide [1]. Surgery resection with a curative intent is the most effective treatment for early stage non-small- cell lung cancer (NSCLC). However, even after complete surgical resection, the rate of recurrence for stages I to III of NSCLC range from 30 to 70% [2] with a high incidence of recurrence during the first 2 years. Based on this concern, published practice guidelines recommend multidisciplinary clinical and radiographic follow up of patients with resected lung cancer [3]. More specifically, the American College of Chest Physicians recommends that in patients who underwent a curative-intent surgical resection of a NSCLC, a chest computer tomography (CT) should be performed every 6 months for the first 2 years after resection and every year thereafter. In many cases, surgical treatment must be combined with adjuvant chemotherapy and/or radiotherapy which can cause inflammation and fibrosis of the mediastinum. Furthermore, most of lung cancer patients are smokers who may have Chronic Obstructive Pulmonary Disease (COPD) or other inflammatory lung disorders which may result in infectious complications. These circumstances such as inflammation, fibrosis and infectious complications could be presented as both lung parenchymal abnormalities and mediastinal nodal enlargement in thoracic CT that can be misdiagnosed as regional recurrence [4]. Thereby, mediastinal and/or hilar nodal enlargement in the thoracic CT during follow up are common features that usually represent a challenge for the clinician. Positron emission tomography with computer tomography (PET/CT) has been used as a diagnostic tool for recurrence, with a high sensitivity value [5, 6]. However, both CT and PET/CT have shown high false-positive rates and therefore histological confirmation is mandatory to rule out regional recurrence.

Mediastinoscopy has been demonstrated to be useful in the assessment of recurrence in patients with previous surgically-treated lung cancer [7]. However, mediastinoscopy becomes more difficult, unsafe and useless after previous thoracic surgery, especially in patients who have been treated with induction radiotherapy. Furthermore, recurrence can lay on hilar nodes that are not amenable for biopsy by means of mediastinoscopy.

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimum invasive procedure currently proposed as the first choice in the mediastinal nodal staging of lung cancer [8]. Adding endoscopic ultrasound fine needle aspiration (EUS-FNA) to EBUS-TBNA has revealed higher diagnostic yield than using EBUS-TBNA alone, due to the complementary access for different nodal stations by each technique [9]. The evaluation of mediastinal nodes from the esophagus using a convex probe EBUS (EUS-B-FNA) associated to EBUS-TBNA has proven to be useful, with figures similar to those using combination of EUS and EBUS-TBNA [10]. The applications of EUS-B-FNA further than lung cancer staging have been barely investigated [11–13].

Some previous studies have demonstrated the usefulness of EBUS-TBNA in the diagnosis of recurrence in patients with previously treated lung cancer [14–18]. However, some of these studies included short series of subjects or included patients not surgically treated. The aim of our study was to evaluate the usefulness of EBUS-TBNA in the diagnosis of locoregional lung cancer recurrence in a larger cohort of subjects entirely composed of surgically-treated patients and describe our initial experience of EUS-B-FNA in this clinical scenario.

Methods

Patients

We conducted a single-center, retrospective study that included all patients with a previous surgically-treated lung cancer who were referred to our bronchoscopy unit after suspicion of locoregional recurrence from January 2006 to October 2014. Recurrence suspicion was based on hilar or mediastinal lymph node enlargement on CT scan (>10 mm in the short axis on CT) and/or abnormal nodal fludeoxyglucose (FDG) avidity on PET-CT during follow up with or without pulmonary node/s or mass/es. The medical records of all patients were reviewed and clinical characteristics were introduced in a database.

EBUS-TBNA

EBUS was performed at an out-patient setting using a flexible bronchoscope [BFUC180F, Olympus Optical Co Ltd., Tokyo, Japan] with a distal probe capable of producing linear parallel scans of both mediastinal and peribronchial tissues also a working channel suited for the performance of TBNA under direct ultrasound guidance. Local anesthesia and sedation were achieved using topical lidocaine spray and intravenous midazolam, propofol and/or fentanyl in accordance with the standard recommendations [19]. Identified mediastinal and lobar nodes with short-axis diameter of 5 mm or more were targeted under direct ultrasound visualization with a 22-gauge cytology needle specially designed for EBUS-TBNA [NA-201SX-4022, Olympus Optical Co Ltd.]. After passing through the bronchoscope channel, the needle was pushed out of the sheath and inserted into the tracheal or bronchial wall under ultrasound guidance. At the target tissue, the needle tip was located, and then it was pushed forth and back with application of negative pressure using a syringe (with 10-mL suction) connected to the proximal end of the catheter. Finally, the suction was ceased while withdrawing the needle out of the target structure. Samples were labeled according to their

origin, whether it was a normal node showing lymphocytic cells and no neoplastic cells, or a metastatic node showing neoplastic cells. Aspirates containing only isolated dysplastic, bronchial, esophageal or blood cells *or* necrotic tissue were considered inadequate. Nodal sampling was targeted and nodes with high suspicion of malignancy (enlarged on CT scan and/or abnormal FDG avidity) were firstly sampled. If rapid on site examination confirmed malignancy the procedure was then finished. In case of a non malignant result, suspicious nodes were sampled three times before ruling out malignancy and a complete systematic sampling (including both lower paratracheal, and subcarinal stations) was performed.

EUS-B-FNA

In cases with lesions that were inaccessible through EBUS-TBNA (nodal stations 5, 8 or 9) the patient underwent directly EUS-B-FNA. In cases with lesions accessible through EBUS-TBNA the patient firstly underwent EBUS-TBNA. If the lesion was partially visible through EBUS-TBNA and located in a station accessible through EUS-B-FNA then the patient underwent EUS-B-FNA in a single-session procedure. EUS-B-FNA was performed guiding the bronchoscope through the pharynx and advanced into the esophagus under gentle pressure. The sampling method did not differ to that previously described for EBUS-TBNA.

Pathology

The aspirated material in the needle was recovered and the specimens were placed on slides and fixed with 95% ethanol. The slides were stained for one minute with haematoxylin for rapid on-site evaluation. Papanicolau staining with orange A and eosin was done later in the pathology laboratory. The cytologist classified satisfactory nodal samples as "normal tissue negative for malignancy" when the sample contained 40 lymphocytes per high-power field in cellular areas of the smear and/or clusters of pigmented macrophages and no neoplastic cells, or as "metastatic" when recognizable groups of malignant cells were present [20]. Nodes containing only isolated dysplastic, bronchial, esophageal or blood cells or necrotic tissue were considered as non-representative of the targeted structure, and were classified as inadequate. Cell blocks were obtained and processed from the specimens recovered whenever extra material was available after the preparation of a minimum of four slides.

Definitions

Cases in which EBUS-TBNA demonstrated malignant nodes with the same histology as the previous treated lung cancer were considered as recurrence (true positive) and no confirmatory tests were required. Cases in which EBUS-TBNA demonstrated specific benign diseases were considered as true negative and no confirmatory tests were carried out. Cases where EBUS-TBNA demonstrated lymphocytes without malignant cells (negative EBUS-TBNA) underwent confirmatory surgery as a "gold standard" or radiological follow up. Negative EBUS-TBNA was considered true negative if the surgical procedures did not demonstrate nodal malignancy or if remained stable during radiological follow up for 12 months. Negative EBUS-TBNA were considered false negative if the surgical procedures demonstrated nodal malignancy or if radiological progression was proved by CT. Patients that presented with extrathoracic progression that required chemotherapy and/or could not complete 12 months of radiological follow up were also considered false negative. Although diagnostic yield of EBUS-TBNA for global malignancy included patients with metachronous tumours, outcomes of these patients were not used in evaluating diagnostic yield of EBUS-TBNA for locoregional recurrence. Metachronous tumours were defined by Martini et al. [21] as follows: 1) Different histological type from the primary tumour or 2) Same histological type if: a) Free interval between tumours is at least 2 years or b) Origin from carcinoma in situ or c) Second cancer in different lobe or lung, but: i) No carcinoma in lymphatics common to both, ii) No extrapulmonary metastases at time of diagnosis.

Statistical analysis

Data was entered into a database and analyzed using SPSS software, version 18.0 [Chicago, IL, USA]. Categorical variables were expressed as absolute and relative frequencies, continuous variables as means and standard deviations (SD) and non-normally distributed data as medians and interquartile ranges (IQR). The diagnostic sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy of EBUS-TBNA were calculated according to standard definitions for diagnosis of both locoregional recurrence and global malignancies, including metachronous tumors, in patients with previous surgically-treated lung cancer.

Results

Seventy-three patients were included. Patient characteristics are shown at Table 1. Previous histological subtypes of lung cancer were predominantly squamous cell-carcinoma and adenocarcinoma and the majority of patients had undergone previous lobectomy. Most of the patients had a previous systematic mediastinal nodal dissection; the median number of dissected nodes was 14.5 per patient. Six patients had a previous staging mediastinoscopy and 20 patients had undergone EBUS-TBNA before surgery. Primary therapy for lung cancer included surgical resection alone in 47 patients; surgical resection followed by chemotherapy in 16; surgical resection followed by

Table 1 Patients' characteristics

Gender: Male (67/73 (91.7%))
Age: Mean 69 (SD ± 10.4).
Time to recurrence: 23 (IQR: 11.5–49).
CT findings:
Isolated mediastinal lymphadenopaties: 50 (68.5%)
Mediastinal lymphadenopaties & lung nodules/masses: 23 (31.5%)
Stage:
IA: 26 (35.6%)
IB: 17 (23.3%)
IIA: 12 (16.4%)
IIB: 13 (17.8%)
IIIA: 4 (5.5%)
IV: 1 (1.4%)
Surgical treatment:
Lobectomy 48 (65.7%)
Bilobectomy 3 (4.1%)
Neumonectomy 8 (11%)
Wedge resection 14 (19.2%)
Systematic mediastinal nodal dissection:
Linfadenectomy: 58 (79.5%)
Nodes diseccted: 14.5 (IQR: 11–20.75)
Stations diseccted: 4.5 (±1)
Previous mediastinoscopy: 6 (8.2%)
Previous EBUS: 20 (27.4%)
Previous treatment:
Surgery 47 (64.4%)
Adyuvant chemotherapy 16 (21.9%)
Adyuvant radiotherapy 1 (1.4%)
Trimodal treatment 9 (12.3%)

radiotherapy in one patient and induction chemo radiotherapy followed by surgical resection in 9 patients. One patient was diagnosed as stage IV but could benefit for surgical treatment after resection of the single extrathoracic metastasis. The median time between primary surgical treatment and recurrence suspicion was 23 months. CT findings were mediastinal nodal enlargement alone in 50 patients and mediastinal nodal enlargement with lung nodes/masses in 23 patients.

EBUS-TBNA confirmed malignancy in 40 patients while in the remaining 33 did not show malignancy (Fig. 1). The entire 40 patient with malignant nodes were presented with the same histological type as the previous treated lung cancer. However, six patients were considered as metachronous disease instead of recurrence. Of the 34 patients confirmed to have recurrence by EBUS-TBNA, 15 patients underwent chemotherapy, 3 radiotherapy, 14 concurrent chemoradiotherapy and 2 patients received best supportive care. In most of the cases, nodal recurrence affected ipsilateral hilar or mediastinal nodes and, in 20% recurrence affected contralateral mediastinum (N3). Eleven malignant lymph nodes (8 hilars, 3 paraesophageal stations), collected from 10 patients who resembled 29.4% of all recurrent patients diagnosed by EBUS-TBNA, were out of the reach of mediastinoscopy.

Of the 33 patients with non-malignant EBUS-TBNA, two had a specific non-malignant disease diagnosed by EBUS-TBNA (1 nodal tuberculosis, 1 foreign body reaction). Of the other 31 patients, 26 patients underwent radiological follow up and 5 patients underwent surgery. Of the 5 patients that underwent surgery one patient with isolated lymphadenopathies underwent mediastinoscopy which showed normal lymph tissue and thereby was considered as true negative; the other 4 patients that presented node or mass associated to the nodal enlargement underwent lobectomy: 3 patients had a final diagnosis of metachronous lung cancer without nodal involvement while one other patient presented a N1 positive interlobar node in the lobectomy resection sample and thus was considered as a false negative of the EBUS-TBNA.

Of the 26 patients who had radiological follow up 18 patients remained stable after 12 months, three presented with thoracic radiological progression and 5 patients presented with extrathoracic progression and died before completing 12 months of follow-up and thereby were considered false negative.

Seven patients underwent EUS-B-FNA because the lesion was difficult to be accessed (one 2R station node, one 4 L station node) or inaccessible for EBUS (five paraesophageal (8) stations) (Table 2, Fig. 2). Of the 2 patients with a lesion accessible by means of EBUS-TBNA both had an EBUS-TBNA before the EUS-B-FNA in a single-session procedure while the 5 patients with lesions not reachable by means of EBUS-TBNA directly underwent EUS-B-FNA. EUS-B-FNA confirmed the recurrence in four cases. There were no major complications related to EBUS-TBNA.

The sensitivity, specificity, negative predictive value, positive predictive value and overall accuracy of EBUS-TBNA for the diagnosis of locoregional recurrence in patients with previous surgically-treated lung cancer were 80.9, 100, 69.2, 100 and 86.6% respectively. The sensitivity, specificity, negative predictive value, positive predictive value and overall accuracy of EBUS-TBNA for the diagnosis of global malignancy, including metachronous tumours, in patients with previous surgically-treated lung cancer were 81.6, 100, 72.7, 100 and 87.6% respectively.

Discussion

Locoregional recurrence represents a significant problem in lung cancer, which corresponds to a quarter of recurrences after surgery [22]. Reported rates of locoregional

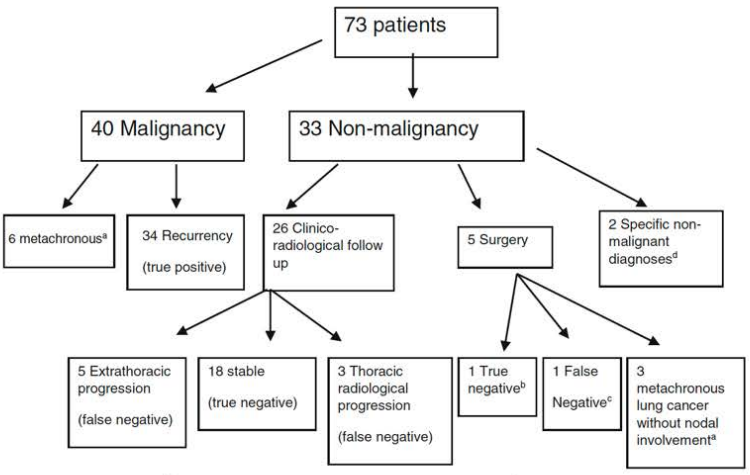

Fig. 1 [a]Excluded for analysis of recurrence. [b]Mediastinoscopy showing normal lymph tissue. [c]One patient with malignant N1 interlobar node in the resection specimen. [d]One tuberculosis, one foreign body reaction

recurrence of NSCLC after surgery vary widely in literature due to the heterogeneity of the studies, as some of them including small sample sizes, variable disease stages, differences in follow up time and unclear definitions of recurrence versus metachronous tumours. Locoregional recurrences typically occur rapidly, as nearly 90–95% of all local recurrences develop during the first five years after the initial surgery [23]. Pathological stage, surgical technique (wedge resection or segmentectomy versus full lobar resection) and patients' characteristics have been demonstrated to be independent predictors of recurrence. Although postoperative surveillance programs have not established a survival benefit, most guidelines recommend follow up of patients after curative-intent surgery. In our series, half of the patients were presented with nodal local recurrence, 6.8% extrathoracic metastases and 12% metachronous lung cancer, confirming that the likelihood of malignancy in these patients is high and thus the diagnosis and clinical-decision making cannot rely only on image-based explorations.

Table 2 EBUS procedure

Total nodes sampled: 213	
Nodes sampled (per patient): 2.92 (SD ± 2.2)	
Stations sampled (per patient): 2 (SD ± 1.2)	
Malignant nodes[a]: 45	
Mediastinal: 37	
2 L: 1	2R: 1
4R: 7	4 L: 11
7: 14	
8 L: 2	8 L: 1
Hilar: 8	
10 L: 1	10R: 2
11 L: 2	11R: 3
Mean size[b]: 14.8 (IQR: 11.5–18.4)	
Esophagus (EUS-B-FNA): 7 (9.6%)	
N in recurrence (34):	
N1: 5 (14.7%)	
N2: 22 (64.7%)	
N3: 7 (20.6%)	

[a]In patients with recurrence diagnosed by EBUS-TBNA
[b]In mm (short-axis diameter)

Before EBUS-TBNA, mediastinoscopy was the most used diagnostic method as an invasive approach to explore the mediastinum. However, few studies have focused on the role of mediastinoscopy in the diagnosis of locoregional recurrence. The concept of "complex" mediastinum refers to an altered fibrotic mediastinum secondary to previous thoracic surgery (primary mediastinoscopy or nodal dissection) or induction therapy [24]. Although some studies performed by expert surgeons in referral hospitals have shown good accuracy of remediastinoscopy, with complication rates similar to primary mediastinoscopy [25], remediastinoscopy is not widely used due to concerns about safety and usefulness [26]. In our series, one third of the patients had concurrent radiotherapy and/or chemotherapy and 7 patients had a previous mediastinoscopy. Regarding the diagnosis of local lymph node recurrence, only one patient required mediastinoscopy after an EBUS. This particular patient

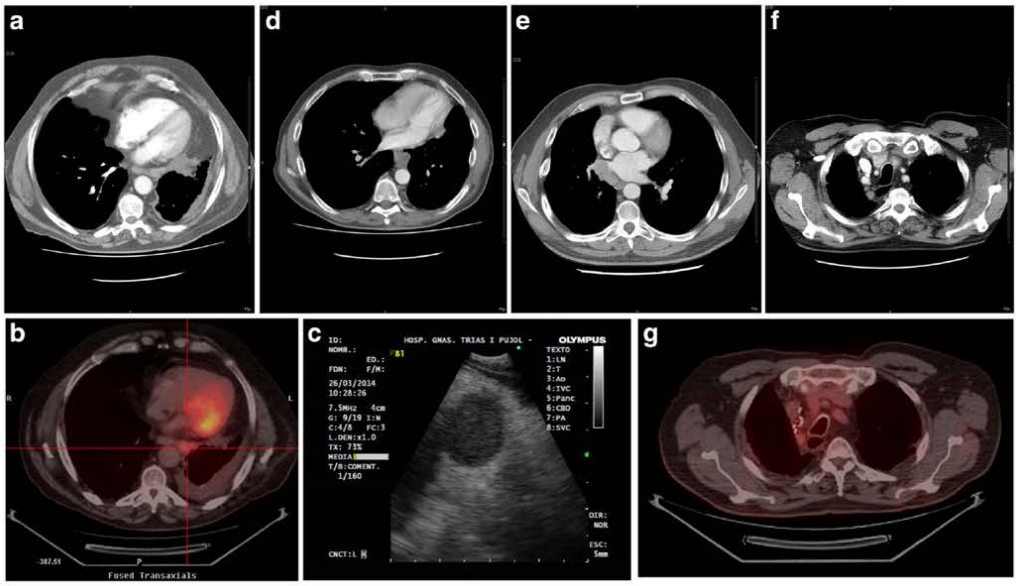

Fig. 2 Four cases of recurrence diagnosed by EUS-B-FNA: *Case 1* (**a**, **b**, **c**): a 68-years old man with a stage IIA squamous-cell carcinoma in LLL treated by means of lobectomy presented a pleural effusion on the first CT control 6 months after the surgery, the PET/CT showed a high uptake on a 8 left station node, an EUS-B-FNA confirmed recurrence. *Case 2* (**d**): a 55-years old man with a previous stage IA NSCLC-NOS in RUL treated by means of wedge resection presented an 8 left lymphadenopathy on the first CT control 6 months after the surgery. *Case 3* (**e**): a 40-years old man with a carcinoid on middle lobe treated by means of RLL and ML bilobectomy presented a mass/nodal enlargement on right 8 station that invaded the pulmonary veins, the diagnosis was consistent with atypical carcinoid. *Case 4* (**f**, **g**): a 71-year old man with a stage IIA adenocarcinoma in RUL treated by means of lobectomy presented a 2R adenopathy in a CT 21 months before the surgery. Although being a paratracheal node the approach was easier through the esophagus

neither underwent previous mediastinoscopy nor received neo/adjuvant therapy. The mediastinoscopy showed no malignancy and the patient remained stable in subsequent radiological follow up. In our series, almost one third of the patients with recurrence confirmed by EBUS-TBNA presented with nodes out of the reach of mediastinoscopy: 3 patients presented with paraesophageal lesions and 7 patients had hilar lesions.

One of the advantages of EBUS-TBNA is its ability to sample hilar nodes. Probably this advantage has not been properly emphasized. One possible explanation is that EBUS-TBNA has been mainly used for mediastinal staging of lung cancer where hilar involvement is not relevant enough since contralateral hilar N3 involvement without N3 mediastinal affection is not very frequent and the distinction between N0/N1, although has importance in the prognosis and the choice of treatment, rarely affects the surgical indication. Nevertheless, the evaluation of N1 nodes is crucial in patients undergoing sublobar resection or local tissue-sparing treatments such as brachytherapy, radiofrequency ablation and stereotactic body radiation. These are the only therapeutic options to many patients who cannot benefit from conventional surgical intervention, including those with previous surgical treatment like in our series. In our study the only surgical false negative case presented with N1 interlobar node. The reported sensibility for EBUS-TBNA in the diagnosis of N1 disease is lower than that described for N2 staging [27], mainly because the size of the convex probe EBUS in many cases does not permit to reach N1 nodes beyond station 11. However, this issue could be solved with the development of new thinner convex probe EBUS. Recently, Wada et al. [28] described their first experience with a new thin convex probe echobronchoscope [BF-Y0046 Olympus Medical Systems Corp] with a thinner tip (5.9 mm) and a larger bending angle than conventional convex probe ecobronchoscopes. These authors reported an improved accessibility to the distal airways in a porcine model, suggesting that thinner echobronchoscopes would increase the diagnostic yield of N1 nodes (especially those beyond the hilum) and peripheral nodes/masses.

Endoscopic ultrasound with a convex probe ultrasonic bronchoscope (EUS-B-FNA) was first described in 2009 [10]. EUS-B-FNA broads the diagnostic yield of EBUS-

TBNA alone because it allows the approach of nodal stations that are not in contact with the tracheobronchial wall (stations 5 and lower mediastinal stations: 8, 9) and also increases the access to nodes that could be better achieved through the esophagus. Moreover EUS-B-FNA is also better tolerated and safer in patients with respiratory impairment. Although these advantages, EUS-B-FNA requires a learning period before systematically employed, as the endobronchial references are lost on the white light image and the ultrasonographic anatomy boundaries vary from the endobronchial view. In our series, ten percent of the patients underwent EUS-B-FNA, and 4 patients (12.9%) with recurrence diagnosed by EBUS-TBNA were exclusively diagnosed by EUS-B-FNA (3 in paraesophageal stations and one in 2R station). In our study, some nodes although located on subcarinal or paratracheal stations, were more easily reached by the esophagus (Fig. 2) due the post-surgical changes in the mediastinal architecture. Few studies have described the usefulness of EUS-B-FNA apart from lung cancer staging. Szlubowski et al. [11] published their experience with EUS-B-FNA combined with EBUS-TBNA in the lung cancer restaging after induction therapy and other two studies described the usefulness of EUS-B-FNA in the sampling of left adrenal gland in patients with lung cancer [12, 13]. To our knowledge this is the first study in which EUS-B-FNA has been used in the diagnosis of locoregional lung cancer recurrence.

Although the interpretation of the visualized mediastinal structures after surgery is more difficult than in a naïve mediastinum, our study demonstrates excellent diagnostic performance of EBUS-TBNA in the diagnosis of locoregional recurrence of patients with surgically treated lung cancer. Our results are similar to those reported for new developed lung cancer staging and also comparable to those to previous studies that included shorter series of surgically-treated patients. Yamamoto et al. [17] in a series of 40 surgically-treated patients showed a sensitivity and NPV of 100%, while Han et al. [16] in a series of 42 surgically-treated patients showed a sensitivity of 94.3% and a NPV of 77.8%. Other studies that included non-surgically-treated patients [14, 15, 18] also demonstrated a high diagnostic accuracy of EBUS-TBNA in the diagnosis of lung cancer recurrence. Thus, changes in the mediastinal anatomy in a post-surgical mediastinum do not affect the diagnostic yield of EBUS-TBNA procedure. Remarkably, in another situation of "complex" mediastinum, following induction therapy, EBUS-TBNA showed lower diagnostic value in restaging of lung cancer [29, 30]. This is probably not due to the procedure itself, but because of complexity in the interpretation of the pathological samples. It has been previously reported that aspirates from malignant nodes treated with chemotherapy may content less cellular burden that could have necrotic tissue, making the pathologic interpretation more difficult.

Conclusions

EBUS-TBNA is proved to be an accurate, safe and minimally invasive procedure in the diagnosis of locoregional recurrence of surgically-treated lung cancer and should be considered as a first choice in patients with radiological abnormalities during follow up. EUS-B-FNA demonstrates to be useful in patients with locoregional lung cancer recurrence, not only in stations not reachable by EBUS-TBNA, but also in paratracheal nodes that could be better attained by the esophagus, whenever there are changes in the normal mediastinal architecture.

Abbrevations

COPD: Chronic obstructive pulmonary disease; CT: Computed tomography; EBUS-TBNA: Endobronchial ultrasound transbronchial-guided needle aspiration; EUS-B-FNA: Endoscopic ultrasound fine-needle aspiration using an echobronchoscope; EUS-FNA: Endoscopic ultrasound fine-needle aspiration; FDG: Fludeoxyglucose; IQR: Interquartilic range; NPV: Negative predictive value; NSCLC: Non-small cell lung cancer; PET/CT: Positron emission tomography/Computed tomography; PPV: Positive predictive value; SD: Standard deviation

Funding

Dr. Felipe Andreo obtained a grant from the Spanish Society of Pulmonology (SEPAR).

Availability of data and materials

The database used for the study can be available from the corresponding author under demand if needed.

Authors' contributions

JSS Designed the study, performed EBUS-TBNA and EUS-B-FNA and wrote the manuscript. PS Designed the study, performed EBUS-TBNA and EUS-B-FNA and wrote the manuscript. FA performed EBUS-TBNA and EUS-B-FNA and revised the manuscript. MT performed EBUS-TBNA and revised the manuscript. CC performed EBUS-TBNA and EUS-B-FNA and revised the manuscript. TM performed follow-up, selected patients and revised the manuscript. EC performed follow-up, selected patients and revised the manuscript. EF performed surgical procedures and revised the manuscript. SGR performed surgical procedures and revised the manuscript. JRM designed the study and revised the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

All the images used as figures in the manuscript were obtained once the patients gave the consent for publication.

Ethics approval and consent to participate

All patients signed the informed consend of the study that was approved by the local ethics committee (Comité de ética Hospital Germans Trias i Pujol).

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Received: 14 December 2016 Accepted: 22 February 2017
Published online: 28 February 2017

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Estudi 3

Identification of oestrogen, progesterone receptor and human epidermal growth factor receptor 2 expression in mediastinal metastases of breast cancer obtained by endobronchial ultrasound-guided transbronchial needle aspiration.

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Cytopathology. 2018 Feb;29(1):35-40.

Factor d'impacte a 2017: 1.376.

ABSTRACT

***Introducció**: En pacients amb càncer de mama, l'expressió de receptor d'estrogen (RE), receptor de progesterona (RP) i expressió del receptor del factor de creixement epidèrmic humà (HER2) són crucials en l'elecció del tractament. A més, l'expressió del receptor pot variar respecte del tumor primari. L'objectiu del nostre estudi va ser analitzar la utilitat de l'ultrasonografia endobronquial amb punció aspirativa transbronquial (USEB-PATB) per obtenir mostres que permetin la identificació dels RE, RP i l'expressió de HER2 en pacients amb metàstasi mediastíniques de càncer de mama.*

***Mètodes**: Es van analitzar retrospectivament les dades clíniques de tots els pacients del nostre centre amb diagnòstic final de metàstasi mediastíniques de càncer de mama diagnosticats per USEB-PATB. Es va calcular la capacitat de l'USEB-PATB per obtenir mostres que permetessin l'estudi de l'expressió de HER2.*

***Resultats**: Es van incloure 24 pacients. Es va poder realitzar l'estudi de RE, RP i HER2 en 22, 20 i 22 pacients respectivament. En 20 dels 24 pacients va ser possible investigar l'expressió dels tres tipus de receptors. Els 4 casos restants, en els que el test d'expressió dels receptor RE, RP i HER2 no es va poder realitzar, va ser degut a manca de mostra. En els casos amb resultats òptims per USEB-PATB i el tumor primari, la concordança va ser major pel RE (16/19) i HER2 (12/14) que pel RP (8/17). Basant-se el l'estat del receptor, es va procedir a un canvi en l'elecció del tractament en 5 pacients.*

***Conclusió**: En pacients amb metàstasi mediastíniques de càncer de mama, l'estudi de l'expressió del RE, RP i HER2 es pot realitzar amb mostres obtingudes per USEB-PATB sempre que la mostra obtinguda sigui suficient.*

Accepted: 30 August 2017
DOI: 10.1111/cyt.12486

ORIGINAL ARTICLE

WILEY

Identification of oestrogen, progesterone receptor and human epidermal growth factor receptor 2 expression in mediastinal metastases of breast cancer obtained by endobronchial ultrasound-guided transbronchial needle aspiration

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Funding information
Catalan Fundation of Pulmonology

Background: In breast cancer patients, the expression statuses of oestrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) are crucial in the choice of treatment. Receptor expression in metastatic lesions can differ from the primary tumour. The aim of our study was to analyse the utility of endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) to obtain samples allowing the identification of ER, PR and HER2 expression in patients with mediastinal metastases of breast cancer.

Patients and methods: The clinical files of all patients with a final diagnosis of breast cancer mediastinal metastases diagnosed by EBUS-TBNA in our institution were retrospectively analysed. The ability of EBUS-TBNA to obtain samples that allowed hormone receptor and HER2 expression analysis was calculated.

Results: Twenty-four patients were included. ER, PR and HER2 assessments could be performed in 22, 20 and 22 patients, respectively. In 20 of the 24 patients it was possible to investigate all three types of receptor expression. In the remaining four cases, where ER, PR or HER2 expression tests could not be performed, it was due to a lack of tissue. In cases with adequate results for EBUS-TBNA and the primary tumour agreement was greater for ER (16/19) and HER2 (12/14) than PR (8/17). Based on receptor status, there was a change in the choice of treatment for five patients.

Conclusion: In patients with breast cancer mediastinal metastases, ER, PR and HER2 expression can be assessed in samples obtained by EBUS-TBNA whenever a sufficient tissue sample is collected.

KEYWORDS
breast cancer, endobronchial ultrasound, HER2, oestrogen receptor, progesterone receptor

1 | BACKGROUND

The expression statuses of oestrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) are clinically relevant for the selection of targeted therapy and the definition of clinical outcome in patients with early and advanced breast cancer. In a metastatic setting, the receptor status can differ from the primary tumour in up to 40% of cases.[1-4] The molecular

characterisation of metastatic breast cancer may lead to changes in the treatment choice and is associated with prognostic impact.[5] Accordingly, international practice guidelines recommend biopsy of metastatic sites whenever possible.[6]

Thoracic metastases are common in breast cancer, whether in the form of pulmonary nodules or mediastinal nodes. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a relatively novel technique whose use has become widespread for the diagnosis and staging of lung cancer. Furthermore, EBUS-TBNA has proven to be useful in diagnosing mediastinal relapse in patients with extrathoracic malignancies.[7] EBUS-TBNA can obtain high-quality specimens that allow immunohistochemical staining and molecular analyses.[8,9] In a previous study,[7] we described our initial experience with EBUS-TBNA samples for the identification of ER, PR and HER2 expression in patients with mediastinal metastases of breast cancer. After this previous study was published, ER and HER2 analysis was carried out in some of these cases at the request of the oncologists. In this present study, we present full results with additional new cases. Moreover, we evaluated the influence of EBUS-TBNA results on clinical decision making (measured as treatment choice changes).

2 | METHODS

2.1 | Patients

We retrospectively analysed the clinical files of all patients with a final diagnosis of mediastinal metastases of breast cancer obtained by EBUS-TBNA from December 2009 to September 2016. The clinical suspicion of mediastinal nodal metastases was based on nodal enlargement (short axis >10 mm) in computer tomography (CT) (with or without lung lesions) and/or on 2-fluoro-2-deoxy-D-glucose uptake in positron emission tomography-CT during follow-up. Patient characteristics including histological subtype, CT findings, site of relapse (only intrathoracic or intra- and extrathoracic), time to relapse and primary tumour ER, PR and HER2 status were recorded. All patients gave informed consent before the EBUS-TBNA procedure.

2.2 | EBUS-TBNA procedure

EBUS-TBNA was performed in an outpatient setting using a flexible bronchoscope (BF-UC160F-OL8; Olympus Optical Co. Ltd., Tokyo, Japan) with a distal probe capable of producing linear parallel scans of the mediastinal and peribronchial tissues and a working channel suited to the performance of TBNA under direct ultrasound guidance. Local anaesthesia and conscious sedation were achieved using topical lidocaine spray and intravenous midazolam, respectively[10]. Mediastinal and hilar nodes with a short-axis diameter of 5 mm or more identified during the procedure were sampled by direct ultrasound visualisation with a 22-gauge cytology needle specially designed for EBUS-TBNA (NA-201SX-4022; Olympus Optical).

2.3 | FNA

The aspirates were recovered and placed on slides, fixed with 95% ethanol and stained with haematoxylin for rapid on-site evaluation by a cytopathologist. The Papanicolaou technique was completed later in the pathology laboratory. Cell blocks were prepared using additional slides to those previously stained with haematoxylin. Slides were slightly air-dried to clot that were scraped into 10% neutral buffered formalin where they were fixed for a minimum of 6 hours. Blocks were embedded in paraffin and sectioned (4-μm thickness). Routine haematoxylin-eosin staining was used on the cell-block sections.

2.4 | Immunohistochemistry (IHC)

Immunostaining was performed on 4-μm cell-block sections using the antibodies against ER (6F11 clone, dilution 1:40, Novocastra; Newcastle upon Tyne, UK) and PR (16 clone, dilution 1:40; Novocastra) separately. The percentage of tumour cells that showed positive staining for ER and PR and the intensity of the staining were recorded. Interpretation of the assay was based on current guidelines[11]. Thus, samples were labelled receptor-positive if a minimum of 1% of tumour cells were positive for ER/PR and as receptor-negative if the specimen exhibited <1% of tumour cells staining for ER or PR at any degree of intensity.

For HER2 detection, staining was performed using the Herceptest Kit (4B5 clone, prediluted; DakoCytomation, Carpinteria, CA, USA) according to the manufacturer's instructions. HER2 results were reported according to the ASCO/CAP guidelines[12] for HER2 overexpression as follows: positive (IHC 3 +), circumferential membrane staining that is complete and intense; equivocal (IHC 2 +). incomplete and/or weak/moderate and within >10% of the invasive tumour cells or complete and circumferential membrane staining that is intense but within ≤10% of the invasive tumour cells; negative, incomplete membrane staining that was faint/barely perceptible and within >10% of the invasive tumour cells (IHC 1 +) and no staining observed; or membrane staining that was incomplete and faint/barely perceptible and within ≤10% of the invasive tumour cells (IHC 0).

In addition to the immunohistochemical assessment of ER, PR and HER2, in patients who had extra specimen material, the immunohistochemical expression of GATA binding protein 3 (L50-823 clone, prediluted, Ventana, Tucson, AZ, USA) and thyroid transcription factor-1 (8G7G3/1, dilution 1:100, DakoCytomation, Carpinteria, CA, USA) were retrospectively measured to confirm a breast origin for the tumour.

2.5 | Fluorescence in situ hybridisation (FISH) for HER2 amplification

HER2 positive cases were confirmed by FISH (Vysis PathVysion, Downers Grove, IL, USA). Following ASCO/CAP guidelines, a FISH *amplified* result was considered positive when there was a ratio of HER2 to chromosome enumeration probe 17 of ≥2.0; with an

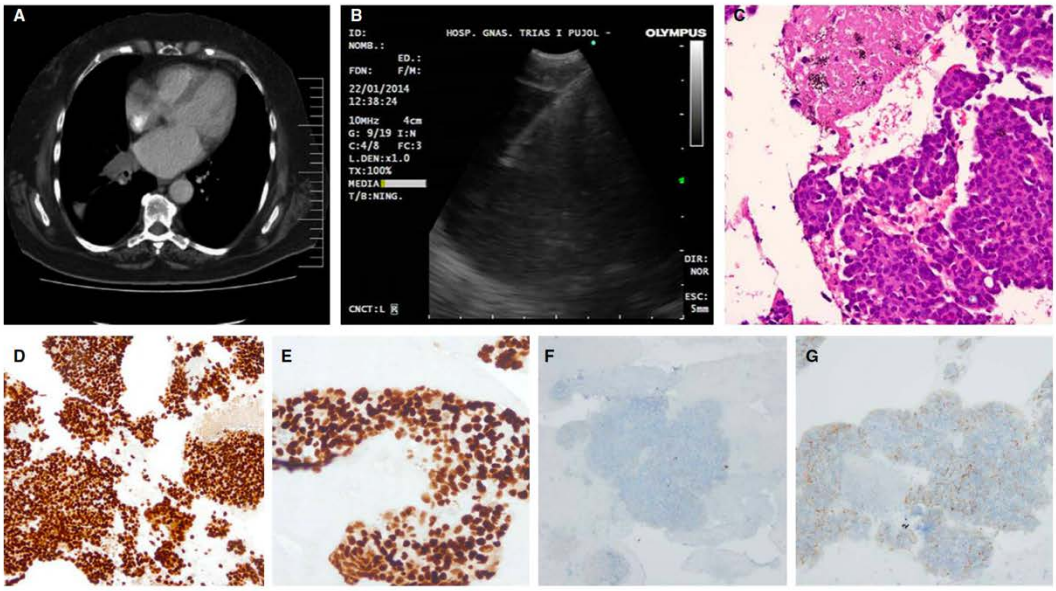

FIGURE 1 A 66-year-old patient with a previous surgically treated oestrogen receptor-, progesterone receptor- and human epidermal growth factor receptor 2-positive primary breast carcinoma presented an enlarged right hilar node in a control computer tomography scan (A). endobronchial ultrasound confirmed the enlarged right hilar node, which was sampled by means of transbronchial needle aspiration under endobronchial ultrasound real-time guidance (B). Mediastinal metastatic relapse was suspected based on haematoxylin-eosin cell-block sections (C) and confirmed with GATA binding protein 3 immunohistostaining (D). The hormone profile of the metastasis was positive for oestrogen receptor (E) and negative for both progesterone receptor (F) and human epidermal growth factor receptor 2 (G)

average HER2 copy number ≥4.0 signals per cell, or a ratio ≥2.0 with an average HER2 copy number <4.0 signals per cells, or a ratio >2.0 with an average HER2 copy number ≥6.0 signals per cell; a ratio <2.0 with an average HER2 copy number ≥4.0 and <6.0 signals per cell was considered equivocal; a ratio of <2.0 with an average HER2 copy number <4.0 signals per cell was considered negative.

The pathological testing of the surgical samples of the primary breast carcinoma of patients treated in our institution did not differ from the testing reported for the EBUS-TBNA specimens. In patients treated for primary breast carcinoma at other institutions, we could not confirm that the pathological testing of the samples was similar to our institution.

2.6 | Statistical analysis

Data were introduced in a database and analysed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). The results were expressed as absolute and relative frequencies for categorical variables and as the means and standard deviations or, when required, as medians and interquartile ranges for continuous variables. First, the ability of EBUS-TBNA to obtain samples that allowed hormone receptor and HER2 expression analysis was calculated. Second, the percentage of treatment choice changes was estimated.

3 | RESULTS

Twenty-four patients were included. Table 1 shows the patients' characteristics. Almost half of the patients presented with extrathoracic metastases (bone, brain or liver) associated with the thoracic findings. Eighteen patients had isolated mediastinal nodes, and six patients presented with pulmonary nodes/masses associated with mediastinal nodes. Two patients with high blood levels of CA 15-3 and mild nodal enlargement on CT underwent positron emission tomography-CT; both presented high 2-fluoro-2-deoxy-D-glucose uptake of mediastinal nodes. A total of 50 malignant nodes were sampled (Table 2). No complications during or after the procedure were recorded. Primary tumour ER, PR and HER2 status was available in 21, 21 and 16 patients, respectively, while in the remaining patients, this information was unknown because they had been previously treated in a different institution.

In nodal metastases, ER, PR and HER2 assessments could be performed in 22, 20 and 22 patients, respectively. In all patients, at least one receptor expression assessment was performed (22 patients underwent ER analysis and the other two patients without ER analysis underwent PR determination). In 20 of the 24 patients, the expression of all three hormonal receptors could be investigated, while in the remaining four cases where ER, PR or HER2 expression could not be determined, this inability was due to a lack of tissue

TABLE 1 Patient characteristics (*n*=24)

Characteristic	*n*
Age (years), mean (standard deviation)	63.57 (0.79)
Time to relapse (months), median (interquartile range)	67 (38-125)
Histological subtype	
Lobular	1
Ductal	19
Non-specified	4
Intrathoracic/extrathoracic spread	13/11
Computer tomography findings	
Nodal enlargement without lung lesions	18
Nodal enlargement with pulmonary nodules/masses	6

TABLE 2 Characteristics of confirmed malignant nodes (*n*=50)

Characteristics	*n*
Location	
Hilar	11
Right (station 11R)	6
Left (station 11L)	5
Mediastinal	39
Upper left paratracheal (station 2L)	2
Lower left paratracheal (station 4L)	2
Lower right paratracheal (station 4R)	17
Subcarinal (station 7)	18
Size[a] M (interquartile range)	14.9 (7.5-21.5)

[a]Short-axis diameter (in mm).

rather than any technical problems. The rates of agreement in receptor status between primary tumour and mediastinal metastases are summarised in Table 3.

ER expression could be compared in 19 cases: 16 were concordant while three had expression on EBUS-TBNA but not the original tumour. PR expression could be compared in 17 cases: eight were concordant while three had expression on EBUS-TBNA but not the original tumour and six had the reverse. HER2 could be compared in 14 cases: 12 were concordant while two had expression on EBUS-TBNA but not the original tumour. Thus, discordance was more frequent with PR expression. The choice of treatment was changed for five patients based on hormone receptor status: three patients with gains in the ER expression received anti-oestrogen treatment (aromatase inhibitors) instead of chemotherapy and two patients with gains in the HER2 expression could benefit from targeted anti-HER2 treatment (trastuzumab) associated with chemotherapy.

Four patients presented a metastasis surgically treated or biopsied in the 18 months before or after the performance of EBUS-TBNA. In these cases (one liver metastasis, one pulmonary metastasis, one brain metastasis and one bone metastasis), the hormonal status did not differ from the EBUS-TBNA results (except for the bone metastasis, where HER2 could not be assessed due to decalcification).

TABLE 3 Receptor expression status and discordance between primary tumor and mediastinal metastases

		Original tumour		
		Positive	Negative	Unknown/not done
ER				
EBUS	Positive	11	3	3
	Negative	-	5	-
	Not done	-	-	2
PR				
EBUS	Positive	1	3	1
	Negative	6	7	2
	Not done	-	-	4
HER2				
EBUS	Positive	-	2	2
	Negative	-	12	6
	Not done	-	-	2

ER, oestrogen receptor; EBUS, endobronchial ultrasound; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2.

It was possible to retrospectively conduct GATA binding protein 3 and thyroid transcription factor-1 expression analysis for the 18 patients for whom sufficient tissue was available (Figure 1). All of them expressed GATA binding protein 3, while none expressed thyroid transcription factor-1.

4 | DISCUSSION

Our study confirms that high-quality tissue specimens can be obtained by EBUS-TBNA to allow analysis of ER, PR and HER2 status in patients with mediastinal metastases of breast cancer. In fact, in our series, the evaluation of at least one receptor expression was possible in all patients, while in most of the patients, all three receptors could be analysed. This information about receptor status led to a change in treatment choice in five patients.

Although international practice guidelines recommend the biopsy of metastatic sites of breast cancer, especially when they represent the first recurrence of disease and/or the receptor status of the primary cancer is unknown, the biopsy of suspected breast cancer metastases is not widely performed in routine practice. Thus, in some institutions, patients are treated in the metastatic setting without a biopsy and with therapeutic decisions being based on the biological characteristics of the primary tumour. There are several benefits to sampling metastatic sites. First, it can provide pathological confirmation of relapse or an alternative diagnosis (such as a second malignancy or a non-malignant disease). Second, it can allow practitioners to obtain a biological profile of the metastases that may be helpful in tailoring both treatment and prognosis.

The lungs are one of the major metastatic sites for breast cancer,[13] and most patients who die of breast cancer present pulmonary metastases.[14] Metastatic involvement of mediastinal nodes occurs frequently.[15] EBUS-TBNA has long been used for diagnosing and staging lung cancer. However, several recent studies have demonstrated its utility in the detection of thoracic nodal metastases from extrathoracic malignancies.[7] The biopsy of breast metastases is also not widely performed, either because it cannot be performed safely for the patient or because the metastatic lesion is not amenable to a core biopsy or an excision sample. In this scenario, fine needle techniques have proven to be very helpful. In particular, EBUS-TBNA has been demonstrated to be a simple, safe and accurate procedure that can be performed in an outpatient setting under local anaesthesia and conscious sedation. In our series, almost half of the patients presented with extrathoracic spread (brain, liver or bones) associated with mediastinal metastases, and therefore could benefit from EBUS-TBNA as a safe alternative to surgical procedures. Another reason the sampling of metastatic breast cancer lesions is not widely performed is because it has been claimed that discrepancies in receptor expression between primary tumour and metastatic lesions may be the consequence of technical failures rather than biological behaviour, given that the first studies reporting discrepancies were published more than 30 years ago[1–3] and there are serious concerns regarding their methodology. At present, several technical issues that can cause artefacts in hormone receptor expression have been identified. For example, specimens from bone metastases need decalcification, which can cause artefacts in hormonal expression.[16] In such cases, sampling of the mediastinum represents not only a safer choice but also an alternative to avoid this technical pitfall. Other studies have shown discrepancies in hormonal expression between surgical excisional biopsy/core biopsy and FNA samples of the same lesion.[17,18] However, these authors attributed the discrepancies to the fixation method (formalin vs alcohol) instead of the specimen type/sampling method (FNA vs core biopsy), and more recent studies have reported good agreement between immunohistochemical results using cell blocks compared with the corresponding surgical specimens.[19–25] In our study, the hormonal expression in EBUS-TBNA samples could not be compared to core biopsies or excisional samples, since patients with positive EBUS-TBNA results did not undergo confirmatory mediastinoscopy for obvious ethical reasons. However, in four patients who presented a metastasis surgically treated or biopsied in the 18 months prior or after the performance of EBUS-TBNA, full concordance was found between the hormonal expression of EBUS-TBNA samples and the histological specimens.

In our series, in four of 24 patients no assessment of either ER, PR or HER2 expression could be performed. In these cases, this inability was due to a lack of tissue specimen. In our institution, when the first EBUS-TBNA procedures were performed in patients with breast cancer, the single aim of the procedure was to confirm disease relapse, and in some cases, this confirmation was based on cytopathological findings but not on immunohistochemistry. As oncologists, pathologists and pulmonologists became more familiar with the technique, oncologists began to request testing for hormonal receptor expression and pathologists requested that pulmonologists obtain more material during the EBUS-TBNA procedure once relapse is confirmed. In this setting, ER, PR and HER2 assessments were always performed on formalin-fixed, paraffin-embedded cell blocks obtained during EBUS-TBNA at the request of a cytopathologist. Cell blocks in EBUS-TBNA provide histologically alike samples that allow immunohistochemical studies as well as molecular analyses. In lung cancer, there is a wealth of evidence that cell blocks obtained by EBUS-TBNA improve the diagnostic yield of the technique, thus allowing pathological subtyping and molecular analyses.[8,9] Many studies have focused on the usefulness of cell blocks from EBUS-TBNA to detect genetic mutations in patients with locally advanced lung cancer. Moreover, a study by Jennings et al.[26] proved the ability of EBUS-TBNA to obtain tissue samples that were adequate to detect the presence or absence of mutations in the BRAF gene in patients with mediastinal metastatic melanoma. Our study confirms that cell blocks obtained by EBUS-TBNA allow molecular analysis in neoplasms other than lung cancer.

In our study, most results for ER and HER2 were concordant and the only discordance consisted of gains of expression in EBUS-TBNA compared with the original tumour; loss of expression was only seen with PR, which was the most discordant.

5 | CONCLUSIONS

Our study demonstrates that in patients with mediastinal metastases of breast cancer, ER, PR and HER2 expression can be assessed in samples obtained by EBUS-TBNA whenever a sufficient tissue specimen is collected. Based on our results, we recommend that oncologists consider EBUS-TBNA as a first minimally invasive approach in patients with breast cancer mediastinal metastases and as a safe and reliable sampling method in patients with extrathoracic spread. Moreover, we encourage pulmonologists to collect sufficient tissue during the EBUS-TBNA procedure for ancillary ER, PR and HER2 expression analysis.

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How to cite this article: Serra P, Sanz-Santos J, Castellà E, et al. Identification of oestrogen, progesterone receptor and human epidermal growth factor receptor 2 expression in mediastinal metastases of breast cancer obtained by endobronchial ultrasound-guided transbronchial needle aspiration. *Cytopathology*. 2017;00:1–6. https://doi.org/10.1111/cyt.12486

Resum de Resultats

En l'estudi "Systematic compared with targeted staging with endobronquial ultrasound in patients with lung cancer" es van estudiar 107 pacients amb afectació mediastínica N2 per PET-TC (78 N2a i 29 N2b) als que se'ls va realitzar una USEB-PATB de forma sistemàtica, comparant els resultats amb un hipotètic escenari on només s'hagessin puncionat els ganglis patològics per PET/TC.

La USEB-PATB va identificar 3 pacients amb afectació mediastínica N3 (2.8%), 60 amb afectació N2 (18 van ser N2b) (56.1%) i 44 pacients N0/N1 (41.1%). Dels 18 casos de malaltia N2b, 11 eren N2a al PET-TC i haguessin passat desapercebuts en un procediment dirigit. Per tant, la USEB-PATB de forma sistemàtica va aportar informació clínica rellevant en 14 casos (13%): 3 casos de malaltia N3 que haguessin estats diagnosticats com a malaltia N2 i 11 casos amb malaltia N2b que haguessin estats diagnosticats com a malaltia N2a a un procediment dirigit. Dels 44 pacients amb un estadiatge N0/N1 després de l'USEB sistemàtic, 7 van sotmetre's a mediastinoscòpia i 24 a cirurgia directament. La dissecció ganglionar mediastínica quirúrgica demostrà malaltia N2 en 4 pacients, falsos negatius de la USEB-PATB. Dos pacients (1.8%) varen presentar complicacions en relació a l'USEB amb un pneumotòrax i un hematoma mediastínic que no va requerir cap intervenció.

La sensibilitat, especificitat, VPN, valor predictiu positiu (VPP) i exactitud de la USEB-PATB sistemàtica pel diagnòstic de metàstasis mediastíniques de CPNCP va ser del 94%, 100%, 90%, 100% i 96.1% respectivament. Es van demostrar 3 casos de malaltia N3 i 11 casos de malaltia N2b que haguessin passat desapercebuts amb l'estadiatge dirigit al PET-TC.

D'altra banda, en l'estudi "Transbronquial and transesophageal fine-needle aspiration using a single ultrasound bronchoscope in the diagnosis of locoregional recurrence of surgically-treated lung cancer", es van incloure 73 pacients amb diagnòstic de CPNCP (amb predomini de carcinoma escatós i adenocarcinoma) que havien estat tractats amb cirurgia toràcica prèvia, majoritàriament lobectomia (alguns casos de quimioteràpia i radioteràpia

d'inducció afegida) que van ser remesos a la unitat d'endoscòpies per a la realització de una USEB-PATB després de la aparició de d'afectació mediastínica a un TC de control.

La USEB-PATB va demostrar malignitat en 40 pacients (54%), tots ells amb la mateixa histologia que el tumor previ (la majoria en regions hiliars o mediastíniques ipsilaterals, excepte un 20% amb afectació N3). Cal destacar que 11 pacients presentaven afectació ganglionar fora de l'abast de la mediastinoscòpia. En 7 pacients es va realitzar una USE-B-PA per les característiques de l'afectació ganglionar (estacions 8 i 9) o perquè eren molt més fàcilment abordables per via esofàgica (dos casos de estacions 2R i 4R).

Dels 33 pacients sense malignitat per USEB-PATB, 2 presentaven malalties benignes especifiques (un era una TB i l'altre una reacció a cos estrany per material de hemostàsia), 26 es van remetre a seguiment radiològic (només 5 van presentar progressió als 12 mesos) i 5 van ser sotmesos a procediments quirúrgics: 3 a cirurgia per lesions parenquimatoses associades on la dissecció ganglionar demostrà tumor metacrònic sense afectació mediastínica, 1 a mediastinoscòpia que va resultar negativa per malignitat (confirmant el verdader negatiu) i un cas amb afectació N1 hiliar no diagnosticada per USEB-PATB.

La sensibilitat, especificitat, VPN, VPP i exactitud pel diagnòstic de recidiva mediastínica en pacients amb CPNCP prèviament tractat quirúrgicament, van ser del 80.9, 100, 69.2, 100 i 86.6% respectivament, i en cas del diagnòstic global de malignitat (inclosos els casos de tumor metacrònic), van ser del 81.6, 100, 72.7, 100 i 87.6% respectivament.

Finalment, en l'estudi "Identification of oestrogen, progesterone receptor and human epidermal growth factor receptor 2 expression in mediastinal metastases of breast cancer obtained by endobronchial ultrasound-guided transbronchial needle aspiration", es van incloure 24 pacients amb afectació mediastínica per metàstasis de carcinoma de mama (18 d'elles únicament amb

afectació ganglionar) als que es va realitzar una USEB-PATB. Es van analitzar un total de 50 ganglis, sense complicacions en els procediments.

Es va poder analitzar el ER, PR i HER2 en 21, 21 i 16 pacients respectivament. En la majoria de casos es va poder comparar l'expressió dels receptors, mostrant la major concordança el PR. En 5 casos es va poder procedir al canvi de tractament per guanys en l'expressió de l'ER i HER2, beneficiant-se de tractament específic.

A 18 dels pacients dels que disposàvem de material suficient es va poder realitzar de forma retrospectiva l'anàlisi immunohistoquímic amb GATA-3 (GATA binding protein 3) i TTF-1 (thyroid transcription factor-1) per poder confirmar l'origen mamari de tots elles. En tots els casos es confirmà l'origen mamari.

Resum de la Discussió

La Tesi Doctoral *La Ultrasonografia Endobronquial en l'estudi del mediastí neoplàsic* ha demostrat la utilitat de la prova en tres aspectes que es detallen a continuació.

L'estadiatge mediastínic és un pas transcendental en dins de l'estadiatge del càncer de pulmó, que marca el tractament i pronòstic dels pacients. El correcte abordatge del mediastí, per tant, pot significar un pas crític en aquest aspecte.

En el nostre estudi hem examinat la precisió d'un procés d'estadiatge de forma sistemàtica mitjançant EBUS-TBNA (mostrejant tots els ganglis mediastínics ≥ 5 mm, independentment de les característiques de PET-TC) demostrant que en comparació amb el mostreig dirigit (pressa de mostres només de ganglis amb captació al PET-TC) proporciona informació clínica addicional rellevant en pacients amb presència de malaltia N2 en PET-TC. Per tant podem afirmar que aquesta exploració rigorosa és la forma més adequada per dur a terme l'estadiatge mediastínic en pacients amb CPNCP[74].

Les guies revisades de la ESTS per l'estadiatge mediastínic preoperatori del CPNCP recomana que el requisit mínim d'un procediment invasiu ha de ser mostrejar el gangli més gran de les estacions ganglionars 4R, 4L i 7, i els ganglis amb captació patològica de FDG dins d'aquestes estacions[48]. D'altra banda, altres autors afirmen que s'han d'incrementar aquests requisits mínims i que l'objectiu de l'USEB-PATB hauria de ser aproximar-se el màxim possible a la dissecció ganglionar quirúrgica, la qual cosa implicaria mostrejar tots els ganglis mediastínics ≥ 5 mm amb independència del seu comportament al PET/TC[49]. Òbviament, incrementar el mostreig provoca un increment en la precisió diagnòstica de l'USEB-PATB. Malauradament, l'estadiatge sistemàtic (que representa un increment en el temps del procediment i amb l'esforç) no sempre es duu a terme i molts professionals practiquen procediments dirigits[75]. El nostre estudi demostra que, tot i ser més laboriós que un procediment dirigit, en pacients amb malaltia N2 al PET/TC els avantatges d'un procediment sistemàtic son inqüestionables. En una població amb afectació mediastínica N2 en el PET-TC, la probabilitat de metàstasi ocultes és alta i el mostreig limitat als ganglis amb captació patològica de FDG pot provocar una pèrdua d'informació

clínica rellevant en gairebé el 15% dels casos, be sigui no diagnosticant malaltia N2b o infraestadiant la malaltia N3. És conegut que el pronòstic de la malaltia mediastínica N2b té taxes de supervivència més baixes que la malaltia N2a[76]. En aquest aspecte, es demostra que la USEB-PATB de forma sistemàtica permet distingir entre afectació ganglionar única o múltiple i valorar el nombre de ganglis per estació, que només seria comparable a la realització de tècniques altament invasives com la limfadenectomia transcervical, la limfadenectomia mediastínica vídeo-assistida (VAMLA)[77] i la limfadenectomia mediastínica estesa transcervical (TEMLA)[78].

Una altra avantatge de l'estadiatge mediastínic de forma sistemàtica mitjançant EBUS-TBNA està relacionada amb el canvi de paradigma el tractament amb radioteràpia per a pacients amb CPNCP. Tradicionalment, els pacients amb CPNCP han estat tractats mitjançant irradiació nodal selectiva (ENI), una tècnica basada en la irradiació del tumor primari, així com els ganglis limfàtics hiliars i mediastínics ipsilaterals, fins i tot si no hi ha evidència d'afectació clínica d'aquestes estacions ganglionar. Més recentment, en molts centres s'està realitzant una altra tècnica, anomenada radioteràpia per camp d'irradiació (IFRT)[79] que inclou només el tumor primari i les estacions ganglionars afectades clínicament, la qual cosa permet una major dosi de radiació en el tumor primari i un menor risc de toxicitat, amb la mateixa incidència de fracàs que amb l'ENI. Tenint en compte que el PET-TC és l'estàndard actual per a la planificació de radioteràpia de pacients amb CPNCP[80], a la nostra sèrie el 21% dels pacients amb CPNCP escollits per a la IFRT haurien estat infratractats si la planificació de la dosi hagués estat basada només en els resultats de PET-TC i l'USEB-TBNA dirigida.

Procedir directament a la cirurgia després d'una USEB-PATB sistemàtica negativa sense mediastinoscòpia pot ser controvertit. A la nostra sèrie, només set de 44 pacients amb estadiatge N0/N1 per USEB-PATB es van sotmetre a mediastinoscòpia, que no va demostrar metàstasi mediastínica en cap cas, i tampoc la resecció quirúrgica posterior. Aquests resultats indiquen que, fins i tot en una població amb una alta prevalença de malaltia N2, la USEB-PATB sistemàtica pot tenir un VPN elevat. De fet, un recent assaig controlat

aleatoritzat i multicèntric, conclou que la USEB-PATB unida a la USE-PA quan cal, és equivalent a la mediastinoscòpia, estalvia temps i té menys complicacions que la mediastinoscòpia[51], preveient un canvi de paradigma en l'actitud davant l'estadiatge mediastínic.

El nostre estudi demostra que l'estadiatge mediastínic mitjançant USEB-PATB de forma sistemàtica, independentment de les característiques de PET-TC, proporciona informació clínica important addicional en comparació amb el mostreig dirigit als ganglis amb captació patològica de FDG, pel que recomanem l'ús sistemàtic de USEB-PATB en la pràctica clínica habitual.

A més de la importància descrita de l'estadiatge mediastínica del CPNCP, la recidiva local també representa in problema en el CP, ja que correspon a una quarta part de les recidives[81] i el 90-95% es produeixen els primers 5 anys[82]. L'estadiatge al moment del diagnòstic, la tècnica quirúrgica i algunes característiques clíniques dels pacients, s'han demostrat predictores de la recidiva.

A la nostra sèrie, el 50% de les recidives van ser a nivell ganglionar, posant de manifest la importància de l'abordatge mediastínic. Abans de la USEB-TBNA, la eina diagnòstica per al diagnòstic de recidiva mediastínica era la mediastinoscòpia, que en molts casos era una remediastinoscòpia. En el cas de la remediastinoscòpia, tot i que el fet que es tracti d'un mediastí "complex" (mediastí fibròtic després de la dissecció ganglionar o la teràpia d'inducció) en mans expertes i a centres de referència ha demostrat utilitat clínica[83]. No obstant això, l'alta taxa de complicacions fa que aquest procediment no s'utilitzi habitualment[84,85].

En el nostre estudi, la USEB-PATB ha mostrat ser una alternativa molt adequada a la mediastinoscòpia en pacients amb pacients amb CPNCP tractats quirúrgicament i sospita de recidiva ganglionar mediastínica. Només un pacient va ser sotmès a mediastinoscòpia posterior a la USEB-PATB i en aquest cas la mediastinoscòpia no va mostrat malignitat (el pacient es va mostrar estable en el seguiment clínic-radiològic). El nostre estudi demostra

que, en aquest context clínic, la USEB-PATB no només és una alternativa més segura (no es va produir cap complicació) sinó que a més, és més eficaç atès que un terç dels pacients presentaven recidiva a ganglis no abordables per la mediastinoscòpia (para-esofàgiques o hiliars).

L'abordatge de les estacions hiliars mitjançant la USEB-PATB és una de les avantatges de la prova. Potser l'estudi hiliar no s'ha emfatitzat prou en el procés d'estadiatge del CPNCP, però té gran importància en el pronòstic i en l'elecció del tractament: crucial en alguns tipus de resecció, braquiteràpia, radiofreqüència o radioteràpia estereotàctica (SBRT).

L'addició de la USE-B-PA augmenta el rendiment de la USEB-PATB, permetent l'abordatge d'estacions no abordables per USEB-PATB. La utilitat de la USE-B-PA en l'estadiatge i el re-estadiatge del CPNCP[67] així com en l'estudi de la glàndula suprarenal esquerra en pacients amb càncer de pulmó[86,87] ha estat suficientment demostrada, però, fins el moment, cap estudi ha avaluat la utilitat de aquesta tècnica en la sospita de recidiva mediastínica de CPNCP tractat quirúrgicament. En la nostra sèrie, 4 pacients van ser diagnosticats de recidiva exclusivament per USE-B-PA.

El nostre estudi demostra excel·lents resultats de la USEB-PATB en el diagnòstic de recidiva local de pacients amb càncer de pulmó tractat quirúrgicament, amb xifres comparables a les descrites per a la USEB-PATB en l'estadiatge del CPNCP i a estudis previs amb menys pacients, mostrant una alta precisió diagnòstica de la USEB-PATB en el diagnòstic de recidiva local del càncer de pulmó, amb bona sensibilitat i VPN[68,69,88–90]. Només en casos de pacients tractats prèviament amb teràpia d'inducció[91,92], disminueix lleument el rendiment, atesa la dificultosa interpretació de les mostres d'aquests pacients.

D'altra banda, l'afectació pulmonar mediastínica de càncers extra-toràcics, és un problema habitual en la pràctica clínica diària. L'USEB-PATB ha demostrat la seva utilitat en aquest context clínic. El nostre tercer estudi confirma que la USEB-PATB permet obtenir mostres de teixit de gran qualitat, que permeten l'anàlisi de dianes terapèutiques (estudi de receptors de HER2, ER i PR) en

pacients amb metàstasi mediastíniques de càncer de mama. De fet, en la nostra sèrie, va ser possible l'avaluació d'almenys un receptor en totes les pacients, mentre que en la majoria es van poder analitzar tots tres receptors i això va comportar un canvi de tractament en 5 de elles.

Tot i que les guies recomanen la biòpsia de les metàstasi de càncer de mama (sobretot en la primera sospita de recidiva), hi ha molts centres on es tracta directament sense biòpsia, basant-se en les característiques biològiques del tumor primari. La biòpsia de lesions sospitoses de recidiva no només permet confirmar el diagnòstic de recidiva, sinó que a més permet obtenir el perfil biològic de les metàstasi, és clau en l'elecció del tractament i com a marcador pronòstic.

Les metàstasi pulmonars són una de les metàstasis mes freqüents del càncer de mama[93], i habitualment s'acompanyen d'afectació mediastínica. De fet, la majoria dels pacients que moren per càncer de mama presenten metàstasis pulmonars[94]. A la nostra sèrie, la meitat de les pacients, presentaven metàstasis extra-toràciques a més de les mediastíniques (hepàtiques, SNC, òssies..). En aquests casos, la USEB-TBNA ha demostrat ser molt útil en la detecció de metàstasis ganglionars de malalties extra-toràciques[72] i una bona alternativa per evitar la presa de mostres a llocs amb més dificultat tècnica (com per exemple les metàstasis òssies, que precisen descalcificació de la mostra[93]) o amb major taxa de complicacions. Molts estudis han demostrat bona correlació de les mostres citològiques de neoplàsia de mama (ben sigui de tumor primari com de metàstasi) amb mostres quirúrgiques[95–97].
En aquest sentit es molt important l'adquisició de bloc cel·lular durant el procediment. El bloc cel·lular ja ha demostrat un excel·lent rendiment per a l'anàlisi molecular de les mostres en pacients amb càncer de pulmó [56]. El nostre estudi confirma la utilitat del blocs cel·lular obtinguts mitjançant EBUS-TBNA en neoplàsies mes enllà del càncer de pulmó.

Conclusions

1. En pacients amb CPNCP i malaltia N2 al PET/TC, l'estadiatge de forma sistemàtica mitjançant USEB-PATB (punció de tots els ganglis ≥5mm independentment de les troballes del PET-TC), ha demostrat que proporciona informació clínica addicional important en comparació a l'estadiatge dirigit (punció exclusiva dels ganglis amb afectació al PET/TC) i s'hauria de recomanar davant la en la pràctica clínica habitual.

2. La USEB-PATB ha demostrat ser una prova precisa, segura i mínimament invasiva en el diagnòstic de la recidiva local del CPNCP tractat quirúrgicament, podent considerar-se com la primera prova a realitzar en pacients amb troballes radiològiques durant el seguiment.

3. La combinació de la USE-B-PA a la USEB-PATB en el diagnòstic de recidiva mediastínica en pacients amb CPNCP tractats quirúrgicament ha demostrat ser útil no només perquè permet arribar a estacions no abordables per la USEB-PATB sinó també permet la presa de mostra de ganglis paratraqueals que després dels canvis estructurals mediastínics conseqüència de la cirurgia son més accessibles per l'esòfag.

4. En pacients amb metàstasis mediastíniques de càncer de mama, les mostres obtingudes mitjançant USEB-PATB permeten l'anàlisi de receptors hormonals i la determinació del HER2.

Línies de Futur

La Ultrasonografia Endobronquial amb Punció Aspirativa Transbronquial o Transtraqueal (USEB-PATB) és una innovadora tècnica que ha anat incrementant el seu ús en la última dècada. Aquest fet ha comportat múltiples investigacions al voltant la USEB i molts intents d'estandarditzar-ne l'ús en guies de pràctica clínica, fins a convertir-se en tècnica d'elecció en l'estadiatge mediastínica del CP i demostrant la seva utilitat en moltes d'altres indicacions. Aquesta avenços han comportat la necessitat de més estudis per intentar augmentar les indicacions de la prova i consensuar la sistemàtica del procediment. Per tant, encara hi ha camí per recórrer, i la present Tesi Doctoral ha intentat ajudar a donar llum a algunes d'aquestes qüestions.

La sistematització de la USEB-PATB és un dels punts claus del procediment, i té un clar impacte en la precisió diagnòstica de la mateixa, com s'ha pogut comprovar. El nostre estudi aporta informació clínica rellevant sobre aquesta qüestió, quan es parla del mediastí patològic. Tot i les recomanacions, sabem que no en tots els centres es segueix aquesta estratègia[75]. En el futur, estandarditzar de la millor manera possible la tècnica, permetrà millorar-ne el rendiment.

D'altra banda, l'entrada de nous processadors, aportarà millores en les funcions bàsiques de la imatge ultrasonogràfica, però també, introduirà eines innovadores, com la elastografia, que a partir d'uns patrons d'elasticitat/rigidesa intenta valorar la malignitat dels ganglis i que pot ser molt útil en el diagnòstic i estadiatge de malaltia tumoral mediastínica mitjançant USEB-PATB[98]. També l'aparició de nous ecobroncoscopis més fins, permetrà l'abordatge d'estacions ganglionars (lobars i segmentàries) més dificultoses a dia d'avui, i ja algun ha demostrat la seva utilitat en estudi in vitro[99].

En el moment actual, l'obtenció de material suficient mitjançant USEB-PATB és essencial, tant pel diagnòstic de diferents malalties amb afectació ganglionar mediastínica, com per tots els estudis moleculars que es poden realitzar. Garantir la màxima rendibilitat de les mostres és una tasca important en el futur. En aquest aspecte existeixen noves agulles de 19-gauges s'han avaluat

amb bons resultats per l'adquisició de mostres per USEB-PATB[100] i veurem si en un futur podrien esdevenir claus en el procés.

En conclusió, la USEB-PATB molts camps on continuar avançant, amb millores de coneixement, de material i processadors, així com el desenvolupament de noves eines, per ampliar les seves indicacions i millorar les seves prestacions.

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Annex

Altres Publicacions relacionades amb la Tesi

Altres Publicacions del Doctorand relacionades amb la Tesi:

1. Torky M, Andreo F, **Serra P**. *Incidental diagnosis of pulmonary embolism during routine convex endobronchial ultrasound. Respir Investig*. 2018 Jul;56(4):369-370. doi: 10.1016/j.resinv.2018.04.001. Epub 2018 May 4.

2. Fiz JA, Monte-Moreno E, Andreo F, Auteri SJ, Sanz-Santos J, **Serra P**, Bonet G, Castellà E, Manzano JR. *Fractal dimension analysis of malignant and benign endobronchial ultrasound nodes.* BMC Med Imaging. 2014 Jun 12;14:22. doi: 10.1186/1471-2342-14-22.

3. Sanz-Santos J, Cirauqui B, Sanchez E, Andreo F, **Serra P**, Monso E, Castellà E, Llatjós M, Mesa M, Ruiz-Manzano J, Rosell R. *Endobronchial ultrasound-guided transbronchial needle aspiration in the diagnosis of intrathoracic lymph node metastases from extrathoracic malignancies.* Clin Exp Metastasis. 2013 Apr;30(4):521-8. doi: 10.1007/s10585-012-9556-3. Epub 2012 Nov 30.

4. Sanz-Santos J, Andreo F, **Serra P**, Llatjós M, Castellà E, Astudillo J, Monsó E, Ruiz-Manzano J. *False positive endobronchial ultrasound-guided real-time transbronchial needle aspiration secondary to bronchial carcinoma in situ at the point of puncture: a case report.* J Cardiothorac Surg. 2012 Aug 14;7:74. doi: 10.1186/1749-8090-7-74.

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7. Sanz-Santos J, **Serra P**, Andreo F, Llatjós M, Castellà E, Monsó E. *Contribution of cell blocks obtained through endobronchial ultrasound-guided transbronchial needle aspiration to the diagnosis of lung cancer.* BMC Cancer. 2012 Jan 21;12:34. doi: 10.1186/1471-2407-12-34.

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RESPIRATORY INVESTIGATION ▮ (▮▮▮▮) ▮▮▮–▮▮▮

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Letter to the Editor

Incidental diagnosis of pulmonary embolism during routine convex endobronchial ultrasound ☆

To the editors:

Convex-probe endobronchial ultrasound (CP-EBUS) is widely utilized during transbronchial needle aspiration of mediastinal lymph nodes (TBNA), for diagnosing and staging lung cancer. However, the usefulness of CP-EBUS may extend beyond evaluating mediastinal lymphadenopathies. We report a 51 year old male patient with a 30 pack /year smoking history of chronic smoking who presented with a 1-month history of productive cough and grade II dyspnea, according to the modified British Medical Research Council (mMRC) dyspnea scale. The patient was hypoxemic with an arterial partial pressure of oxygen (PaO_2) of 52 mmHg and an oxygen saturation of 89%. Apart from the thoracic symptoms, the patient also presented with hematuria and abnormal kidney function (elevated blood urea nitrogen and serum creatinine). Thoracic radiography and computed tomography (CT) without contrast revealed a left hilar mass with multiple bilateral lung nodules.

Conventional flexible bronchoscopy was performed, which revealed no endobronchial lesions. During CP-EBUS-guided TBNA of the patient's mediastinal and hilar lymph nodes, we noticed a filling defect in the right pulmonary artery (Fig. 1). Samples taken from the mediastinal lymph nodes were positive for adenocarcinoma. After the patient's renal function improved, a single positron emission CT scan (SPECT-CT) confirmed that the intraluminal filling defect in the right main pulmonary artery was a pulmonary embolism (PE). A subsequent duplex scan showed deep venous thrombosis in

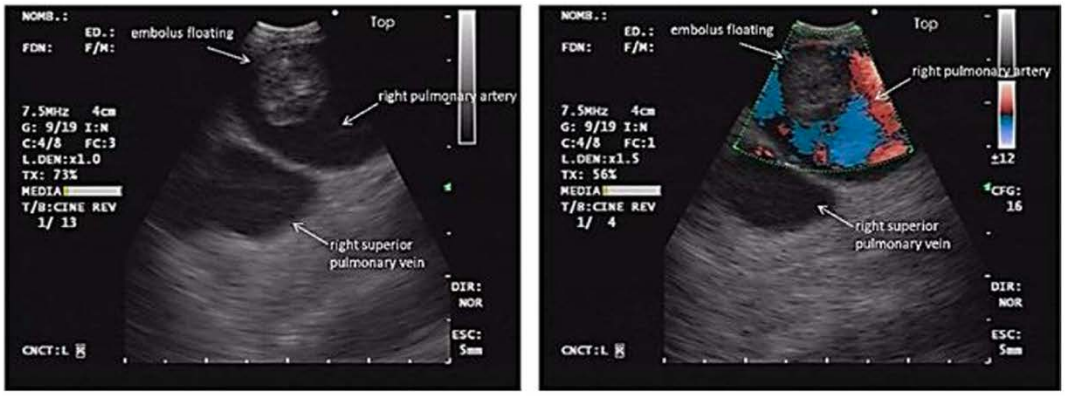

Fig. 1 – CP-EBUS images showing a filling defect within the right pulmonary artery.

☆There was no any financial support or relationships that may pose conflict of interest, The included patient has signed the informed consent of the study.

https://doi.org/10.1016/j.resinv.2018.04.001

the right lower limb. The patient was started on anticoagulation therapy with subcutaneous low-molecular-weight heparin and was transferred to the oncology department.

Cancer patients are at greater risk of developing PE, and the clinical manifestations of thoracic malignancies may interfere with the diagnosis of PE [1]. PE is routinely diagnosed radiographically by CT angiography or ventilation-perfusion scans. However, not all patients are suitable candidates for these imaging techniques, including those with an allergy to contrast or renal impairment. Moreover, some patients may be too hemodynamically unstable to be transferred to radiology units. Due to the anatomical proximity of the airways and pulmonary vessels, clinicians can utilize CP-EBUS to identify thromboses and other pathologies within the central pulmonary branches, although this becomes more difficult in the more distal pulmonary branches. One study found that the accuracy of CP-EBUS in diagnosing central pulmonary emboli that were previously detected by CT angiography was as high as 96% [2].

In our case, the use of contrast imaging was not initially feasible due to the patient's abnormal renal function; therefore, the vascular pathology was first detected by CP-EBUS. A similar case was reported by Sachdeva, et al. who initially detected a PE in the right pulmonary artery while using CP-EBUS to evaluate mediastinal lymphadenopathy [3]. Thus, these two cases highlight the need to routinely examine the pulmonary arteries during CP-EBUS procedures, especially when lung cancer is suspected.

Conflict of interest

The authors have no conflicts of interest.

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30 January 2018

23 March 2018

2 April 2018

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Fiz et al. BMC Medical Imaging 2014, 14:22
http://www.biomedcentral.com/1471-2342/14/22

BMC Medical Imaging

RESEARCH ARTICLE Open Access

Fractal dimension analysis of malignant and benign endobronchial ultrasound nodes

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Abstract

Background: Endobronchial ultrasonography (EBUS) has been applied as a routine procedure for the diagnostic of hiliar and mediastinal nodes. The authors assessed the relationship between the echographic appearance of mediastinal nodes, based on endobronchial ultrasound images, and the likelihood of malignancy.

Methods: The images of twelve malignant and eleven benign nodes were evaluated. A previous processing method was applied to improve the quality of the images and to enhance the details. Texture and morphology parameters analyzed were: the image texture of the echographies and a fractal dimension that expressed the relationship between area and perimeter of the structures that appear in the image, and characterizes the convoluted inner structure of the hiliar and mediastinal nodes.

Results: Processed images showed that relationship between log perimeter and log area of hilar nodes was lineal (i.e. perimeter vs. area follow a power law). Fractal dimension was lower in the malignant nodes compared with non-malignant nodes (1.47(0.09), 1.53(0.10) mean(SD), Mann–Whitney U test $p < 0.05$)).

Conclusion: Fractal dimension of ultrasonographic images of mediastinal nodes obtained through endobronchial ultrasound differ in malignant nodes from non-malignant. This parameter could differentiate malignat and non-malignat mediastinic and hiliar nodes.

Background

The ultrasound technique (US) applies sound waves (1 MHz up to 100 MHz.) that collide with tissues and thus provide energy as images. US has had a wide-ranging impact in medicine due to its low cost and by offering high resolution images.

Ultrasonography endobronchial (EBUS) has been applied as a routine procedure [1]. Three types of EBUS are currently used: EBUS radial ultra-miniature, radial and the convex or curvilinear (CP EBUS). Radial EBUS allows the evaluation of small outlying lung nodules [2]. CP USEB is the most extensively used technique because it allows to carry out mediastinal lymph node puncture (TBNA) [3,4]. Recently, several studies have demonstrated the relation between macroscopic ultrasonographic appearance and vascular patterns [5] and the likelihood of malignancy [6,7]. Although these studies have shown that some features are associated with malignancy, the evaluation of the ultrasonographic appearance depends on the observer subjectivity, and recently one study demonstrated intraobserver and interobserver disagreement [8]. Because the images contain noises mainly as a result of the reflection among adjacent surfaces, it is necessary to process them to be able to separate the real images from the noise.

The present study describes a method that improves the quality of the image, and in consequence the effectiveness of TBNA. The proposed method consists of two sections: one of having the processed image adapted to the specificities of the ultrasonography obtained by means of EBUS that eliminates the devices and specific noise of the application, and a second that characterizes the morphology of the images with the purpose of distinguishing between normal and pathological nodes.

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Fiz et al. BMC Medical Imaging 2014, 14:22
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Material and methods

The study was developed in the Bronchoscopy Dept. of the Hospital Universitary Germans Trias i Pujol and approved by The Human Research and Ethics Committee.

EBUS-TBNA was performed in an outpatient setting using a flexible bronchoscope (BF-UC180F-OL8, Olympus Optical Co Ltd., Tokyo, Japan) with a distal probe capable of producing linear parallel scans of the mediastinal and peribronchial tissues and a working channel suited to the performance of TBNA under direct ultrasound guidance. Local anesthesia and conscious sedation were achieved using topical lidocaine spray and intravenous midazolam, respectively [BTS guidelines]. Mediastinal and lobar nodes with a short-axis diameter of ≥ 5 mm identified during the procedure were sampled under direct ultrasound visualization with a 22-gauge cytology needle specially designed for EBUS-TBNA (NA-201SX-4022, Olympus Optical Co Ltd.). The aspirates were recovered and placed on slides, fixed with 95% ethanol and stained with haematoxylin for rapid on-site evaluation by a cytopathologist. An immediate assessment was given after each pass. Nodes were classified as "normal tissue negative for malignancy" when the sample contained 40 lymphocytes per high-power field in cellular areas of the smear and/or clusters of pigmented macrophages and contained no neoplastic cells or as "metastatic" when recognizable groups of malignant cells were present. Aspirates containing only isolated dysplastic, bronchial or blood cells were considered as inadequate. In these cases the node was punctured as many times as needed to obtain adequate material.

Normal nodes were confirmed to be non-malignant by surgical procedures (patients who underwent mediastinoscopy or thoracotomy with extended nodal dissection) or by clinical and radiological follow-up for at least 18 months. In case of malignant nodes, no further confirmation was performed because the likelihood of false positive EBUS-TBNA results is very low.

Image processing

To improve the quality, we processed the image by means of the following step sequences. The steps are standard image processing procedures that improve the quality of the image [9]. The image was first segmented to select the area of interest. A median filter was applied to remove possible spikes. Afterwards the noise of the image was reduced by a linear average 3×3 low pass filter. Local equalization with structure preserving was applied by means of a histogram 15×15. This last image will be called I1. Then I1 is filtered by means of with two orthogonal Sobel filters, which had the impulsion response (H) of:

$$H = \begin{bmatrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{bmatrix}$$

which yielded images I2 and I3. The Sobel filter was used for enhancing the inner structures of the ganglia.

The final step consisted on combining linearly the filtered images I1, I2 and I3.

Image analysis

The analysis of the images was done by means of two methods: texture and fractal dimension analysis. Texture analysis provides information of the pixels' intensity variability. In areas with soft texture, the range of values around the pixel is small, and when the texture is rough the range is bigger. The texture parameters to analyze are the following: a- Contrast, variance or inertia gives a measure of the intensity between a pixel and its surrounding. For a constant image the contrast is 0. b- Correlation is the relation of a pixel with its surrounding. A constant image has a correlation around 1. c- Homogeneity indicates the degree of vicinity of the elements as well as for intensity of gray. The biggest homogeneity has the value 1.

The fractal dimension of the image was computed by treating the image as a 3D object, and taking horizontal slices of it at different intensity levels. Therefore for each intensity level we created a binary image, where we assigned the value white to the intersection of the surface with the slice and to the inner pixels. In other words, for each gray level we created and image and assigned the white value to the set of pixels with that gray level and the pixels inside the regions. The black value was assigned to the other pixels. The result was that for low values of gray level most of the figure was white, and as the gray level increased, the images begun to take shapes like fiords, as the gray level continued to increase, islands appear, and finally, the whole image finally is black. The algorithm computed the inner area (white space) and its perimeter. We assumed a perimeter model of the node inner structure as follows: Log (Perimeter[n]) = k + α Log(Area[n]). Parameters k and α were computed after a least squares linear regression was applied. The fractal dimension is the α value that models the increase of the perimeter as the area of the figure increases.

A possible characterization of the structures in the images could be done by means of the box counting dimension [10]. We decided not to use it in this problem due to various difficulties.

a) The box counting dimension assumes a binary image with two different zones. The box counting method consists of computing the fractal dimension by counting the boxes that overlap the border between regions at different scales (sizes) of the boxes. This assumes that there is a specific threshold that characterizes the different areas of interest, and the gray level information of the image is lost. In our case, as the different structures of the tissue

are reflected in the intensity (or gray level) of the image, we computed the regression of the log perimeter vs log area, not on the different scales of the boxes, but on the variation of the log area/log perimeter relationship at different gray scale levels.

b) The second difficulty was that the size of the areas of interest in the ultrasonography was small (of the order of less than 100x100, depending on the selected area) and therefore the estimate of the fractal dimension by means of the box counting method would have been unreliable, due to the lack of points.

Statistical analysis of differences in image parameters between independent groups were performed with a Mann_Whitney U test. In addition, a receiver-operator characteristic (ROC) curve was applied to measure the capacity of the method to discriminate between neoplasic and non neoplasic nodes.

The Image processing part of the study was made using the Matlab programming language. The texture parameters were computed by means of the subroutines of the same name (i.e. Contrast, variance or inertia) in the 'Image processing Toolbox', and the fractal dimension part was programmed in Matlab. Statistic analysis was developed with Statistica v.12 (StatSoft, Inc 2013. Tulsa.USA).

Results

Table 1 shows the histological results of 23 biopsied mediastinal nodes. Twelve nodes were malignant.

The ultrasound images were processed in order to improve the quality of EBUS image and to enhance the details, as can be seen in Figure 1 (1-A non processed image, 1-B processed image).

Table 2 shows morphologic parameters and fractal dimension of 23 biopsied lymph nodes. Processed images showed that fractal dimension was lower in neoplasic with respect to non neoplasic nodes. There were no differences between both groups in the morphological parameters.

Figure 2 shows the relationship between the log area and the log perimeter. The slope of the straight line would give us the form in that the log-perimeter grows linearly with the log-area. In this example the relationship agrees with a lineal model. Except for fractal dimension, there were no differences in morphological parameters between images (Table 2). On the other hand, the fractal dimension was smaller in malignant lymph nodes (Mann–Whitney U test for independent groups, $p < 0.05$).

Figure 3 shows the ROC curve of fractal dimension parameter. The area under the curve quantifies the overall ability of the fractal dimension measure to discriminate between neoplasic and non neoplasic nodes. The area under the curve was 0.76 with Std Error of 0.11 ($p < 0.03$).

Table 1 Characteristics and results of lymph nodes

ID	Type	Cytological diagnosis	Type	Station	Size (mm)
1	Malignant	Carcinoma	Breast C	7	23.8
2	"	Carcinoma	Breast C	7	28.9
3	"	Squamous		4 L	8.9
4	"	Squamous		11 L	19.1
5	"	Adenocarcinoma	NSCLC	7	13.2
6	"	Squamous	NSCLC	4 L	6.7
7	"	Adenocarcinoma	NSCLC	4R	9.1
8	"	Squamous	NSCLC	4 L	17.1
9	"	SCLC		4R	11.2
10	"	Adenocarcinoma	NSCLC	4R	13.4
11	"	Adenocarcinoma	NSCLC	7	23.0
12	"	Adenocarcinoma	NSCLC	4 L	9.2
13	Benign	Normal		4R	8.2
14	"	Normal		4 L	4.1
15	"	Normal		7	11.8
16	"	Normal		7	14.2
17	"	Normal		4R	5.9
18	"	Normal		4R	8.3
19	"	Normal		4R	8.6
20	"	Normal		11 L	10.6
21	"	Normal		7	10.4
22	"	Normal		4 L	9.8
23	"	Normal		4R	9.1

Histological characteristics of 23 needled lymph nodes.
NSCLC: non-small cell lung cancer.
SCLC: small cell lung cancer.
Breast C: breast cancer.

Discussion

In this work we studied the relationship between parameters that describe the texture and fractal dimension of endobronchial ultrasonographic images of mediastinal nodes and the likehood for malignancy. In both raw images as well as enhanced ones it was found that there is a statistical difference between malignant and non-malignant nodes in terms of fractal dimension.

The introduction of EBUS-TBNA has provided a significant advance in the staging and diagnosis of lung cancer and other malignancies in a safe and minimally invasive procedure [11]. The analysis of the ultrasonographic appearance of the nodes has been applied to predict malignancy. Fujiwara et al. studied morphologic characteristics of lymph nodes by means of a multivariable analysis that included round shape, distinct margin, heterogeneous echogenicity and presence of coagulation necrosis [6]. The authors found that these morphologic characteristics are independent predictive factors for predicting malignancy. Echogenicity was the parameter

Fiz et al. BMC Medical Imaging 2014, 14:22
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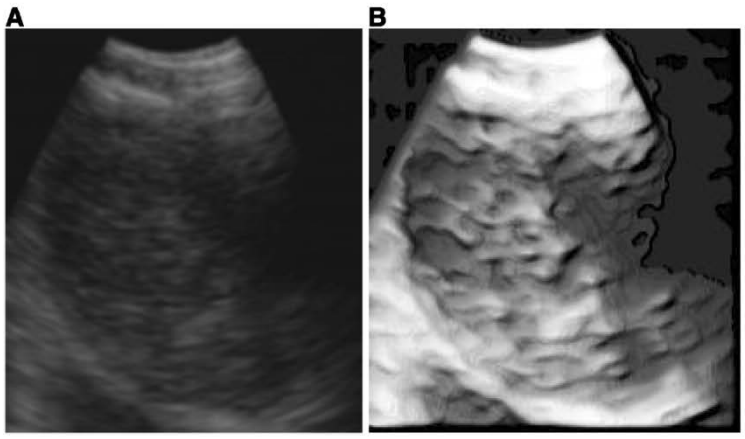

Figure 1 Non processed EBUS image (1-A) and processed image (1-B). Image details are emphasized in image **B** with respect to image **A**.

with the most validated punctuation. The authors did not apply the automatic process of the image, but only qualitative subjective evaluation. Nguyen et al. applied for the first time the second order grayscale texture feature analysis in EBUS [12]. In their study, 52 malignant nodes and 48 benign ones were analyzed. They found that malignant nodes have a higher difference in first and second order texture parameters in relation with benign nodes, using as distinctive features in texture parameters based on first and second order statistics. It should be noted that images were not preprocessed in order to maintain the same real time quality image. On the other hand, the differences in textures after enhancing the image were not significant. This can be attributed to the fact that the processing smoothed the image, eliminated spurious peaks, and enhanced the inner structures of the nodules. This processing that improved the visual appearance of the details of the nodes, changed the texture of the image.

An interesting aspect of the proposal in this paper of introducing the fractal index α, is that this index is complementary with respect to the texture parameters. This complementariness arises from the fact that the fractal index is adapted to the shape of the internal structures of the nodule, and therefore appears as significant after the enhancement of the image. On the other hand the raw image has too much noise, which gives rise to artifacts when computing the fractal index. The fractal dimension is a real number that generalizes the concept of

Table 2 Morphological image parameters

	Processed image		
	Neoplasic	Non Neoplasic	All
Fractal dimension	1.47(0.09)	1.53(0.10)*	1.50(0.10)
Contrast	0.35(0.09)	0.40(0.14)	0.37(0.12)
Correlation	0.95(0.01)	0.95(0.02)	0.96(0.01)
Homogeneity	0.87(0.03)	0.86(0.04)	0.65(0.03)

Mann–Whitney U test for independent groups.
*P < 0.05. Significant difference between neoplasic and non neoplasic nodes.
All values are in mean (SD).
Morphological parameters and fractal dimension of 23 biopsied lymph nodes. The table shows that fractal dimension was lower in neoplasic with respect to non neoplasic nodes. There were no differences in morphology parameters between neoplasic and non neoplasic images.

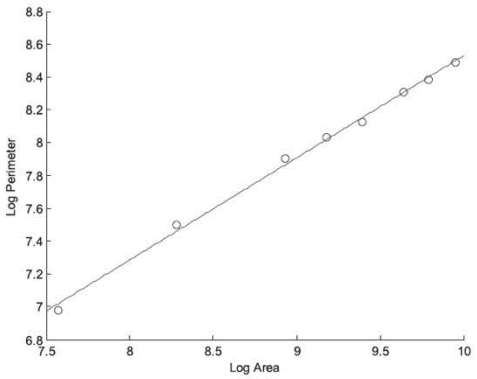

Figure 2 Relationship between the log area and the log perimeter of a node image. The slope of the straight line would give us the form in that the log- perimeter grows linearly with the log-area. In this example the relationship agrees with a lineal model. *Log(Perimeter[n]) = K + αLog(Area[n])*. Coefficients: α = 0.62, K = 2.31.

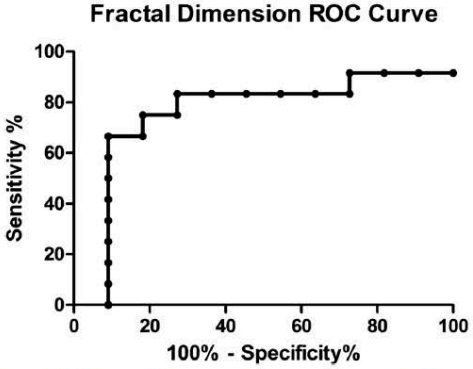

Figure 3 ROC curve of the fractal dimension parameter. The area under the curve was 0.76 ($p < 0.03$).

ordinary dimension for geometric objects. This process also provides data regarding phenomena like deformation, remodeling, breakup and repair. Cancer in general is associated with a disruption of tissue architecture due to the interaction between cells and stroma [13], and fractal-shape parameters could be descriptors of the cell-stroma system. On the other hand, there is a fractal relationship between the degree of apparent heterogeneity of local tissue and the resolution of the measurement, when heterogeneity provides no uniformity in the cell organs examined.

Fractal dimension has been applied in ultrasound echo signals to detect tissue tumors [14,15]. Texture parameters and fractal Higuchi dimension of the ultrasound series detected prostate cancer in small tissue regions with an accuracy of 91% [15]. Zheng et al. [16] applied fractal Brownian motion and k means cluster analysis to detect breast cancer with a recognition rate of 94.5% for malignant tumors. In the present work, we analyzed 23 nodes (12 of them malignant), and applied an algorithm to compute the inner area (white space) and its perimeter. We assume a power model between the perimeter of the inner structure of the ganglia and the area. Difference of fractal dimension between malignant and non malignant nodes was significant, and less in malignant nodes. A possible cause of this slight reduction in fractal dimension of malignant nodes is that cell membranes spread to take the form of a lower energy structure like a circle, therefore, diminishing the fractal dimension of a neoplasic node [13]. In this way, Kikuchi et al. [17] showed that sonography of solid components in cystic epithelial ovarian cancers had a fractal structure, and the mean fractal dimension decreased from 1.26 for serous intracystic components to 1.18 for clear cell adenocarcinoma. In our study the mean fractal dimension was more than 1, meaning the topological line dimension, and it decreased from 1.53 for benign nodes to 1.47 for malignant nodes, the same proportion of the Kikuchi study.

We believe that the principal limitation of our study is the relatively small number of analyzed nodes, but the objective was to describe the fractal nature of the ultrasonographic images of mediastinal nodes. A future application and validation of the present technique could be developed to distinguish between malignant nodes and other non-malignant pathologies that affect mediastinal nodes (such as tuberculosis and chronic inflammatory diseases like sarcoidosis). We should always try to obtain pathological reference diagnosis from suspicious lymph nodes, but in the future, image analysis could assist the bronchoscopist regarding the likelihood to malignancy of the node, as well as the most suspicious region of the node to sample. In consequence, we believe that fractal dimension can constitute a new EBUS parameter to take into account. To our knowledge, this is the first study that applies fractal dimension analysis to EBUS images.

Conclusion

Fractal dimension of ultrasonographic images of mediastinal nodes obtained through endobronchial ultrasound differ in malignant nodes from non-malignant. This parameter could assist the bronchoscopist to differentiate malignant and non-malignant mediastinic and hiliar nodes.

Competing interest

The authors declare that they have no competing interest.

Authors' contributions

JAF: Participated in the design of study, statistical analysis and manuscript writing. EM: Participated in the study design, statistical analysis and manuscript writing, as well as software programming that computed the fractal dimension of the data and the image processing part. FA: Participated in the study design, manuscript writing and bronchoscopy explorations. SJ: Participated in bronchoscopy explorations and contributed in design and manuscript writing. JS: Participated in bronchoscopy explorations and contributed in design and in the writing and revising of the manuscript. PS: Participated in bronchoscopy explorations and contributed in design and revision of the manuscript. EC: Participated in bronchoscopy explorations and in the design and revising of the manuscript. JR: Participated in the design of study, statistical analysis and manuscript writing. All authors read and approved the final manuscript.

Acknowledgments

The authors thank A. Ruiz and A. Barrios for their technical assistance during the performance of the procedures. This work was partially supported by a grant from the Spanish Society of Pulmonology and Thoracic Surgery (grant SEPAR 2010) and by the grand SpeechTech4All (TEC2012-38939-C03-02).

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Received: 22 April 2014 Accepted: 6 June 2014
Published: 12 June 2014

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doi:10.1186/1471-2342-14-22
Cite this article as: Fiz *et al.*: Fractal dimension analysis of malignant and benign endobronchial ultrasound nodes. *BMC Medical Imaging* 2014 **14**:22.

Sanz-Santos et al. Journal of Cardiothoracic Surgery 2012, 7:74
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JOURNAL OF CARDIOTHORACIC SURGERY

CASE REPORT Open Access

False positive endobronchial ultrasound-guided real-time transbronchial needle aspiration secondary to bronchial carcinoma in situ at the point of puncture: a case report

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Abstract

Since the development of endobronchial ultrasound-guided real-time needle aspiration (EBUS-rt-TBNA) no false positive (FP) cases have been described. We present the first FP case for EBUS-rt-TBNA secondary to a carcinoma in situ (CIS) in the bronchial point of puncture. A 66-years-old male was referred to our Institution because of a mass in left lower lobe. The bronchoscopy did not show any endobronchial lesion. The cytology of the washing confirmed an unspecified non-small cell lung cancer. An EBUS-rt-TBNA for staging was carried out. No mediastinal nodes over 5 mm length were found but one single left hilar node at station 11 L was sampled. The cytology of the TBNA showed lymphocytes and neoplastic squamous cells. The patient underwent thoracotomy. On the surgical specimen no metastasis on any of the nodes resected were detected but a CIS on the bronchial resection margin was described. A bronchial biopsy confirmed CIS on the bronchial stump. The reported case depicts an unusual situation, we consider EBUS-rt-TBNA an accurate technique if minimal requirements are met

Keywords: Lung Cancer, Endobronchial Ultrasound, Staging, False Positive, Carcinoma in situ

Background

Endobronchial ultrasound-guided real-time transbronchial needle aspiration (EBUS-rt-TBNA) is a relatively novel technique that has proven useful in lung cancer diagnosis and staging. EBUS-rt-TBNA can be performed under conscious sedation in an outpatient setting. Several studies have demonstrated that EBUS-rt-TBNA is an accurate procedure alternative to surgical staging, with fewer complications and similar figures for sensitivity and specificity. While an average false-negative rate of 10 % represents a handicap for this procedure, no false-positive (FP) cases have been described. We present the first FP case of EBUS-rt-TBNA.

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Case report

A 66-year-old male, with a 40 pack-year smoking history, consulted his general practitioner because of persistent cough lasting for 3 months. A chest x-ray was performed and a mass on left lower lobe (LLL) was detected. The patient was then referred to our Institution. The thoracic CT-scan confirmed the presence of a peripheral mass on LLL (4x3 cm), without evidence of nodal enlargement (Figure 1). A white light bronchoscopy was performed and no endobronchial lesions were detected. The bronchial washing cytology was positive for unspecified non-small cell lung cancer. An EBUS-rt-TBNA for staging was performed. There were no nodes over 5 mm in short-axis diameter on mediastinal stations but one left hilar node, at 11 L station, measuring 12x9 mm was detected and sampled. The cytological examination of the smear showed the presence of lymphocytes and a few groups of neoplastic squamous cells (Figure 2a). The patient was diagnosed with squamous-

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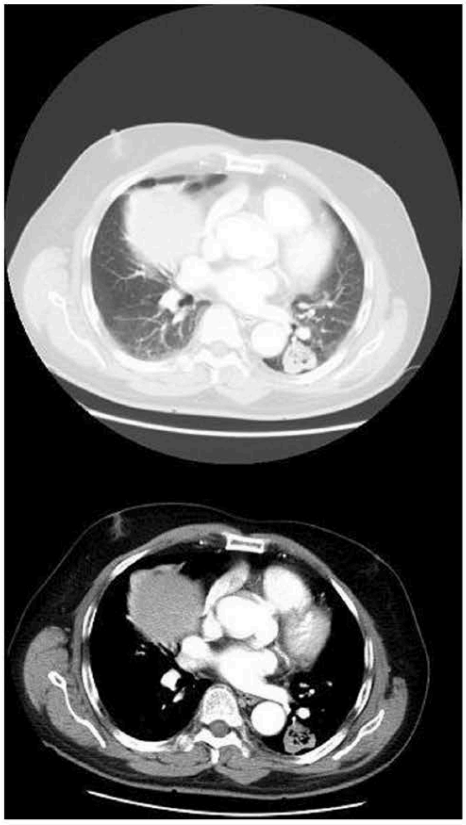

Figure 1 Thoracic CT-scan: Peripheral mass in left lower lobe with central cavitation.

cell carcinoma (SCC) stage IIa cT2aN1M0 and underwent left lower lobe lobectomy. The surgical specimen consisted of a peripheral mass in LLL measuring 4x3 cm consistent with SCC, carcinoma in situ (CIS) on the bronchial resection margin (Figure 2b) without nodal involvement of any of the 9 nodes resected. Several cuts of the hilar nodes were carried out but no neoplastic cells were detected. The postsurgical staging was pT2aN0M0 with CIS on the bronchial resection margin. A few weeks later a bronchoscopy with autofluorescence was performed. An area of low autofluorescence extending from the lobectomy stump to the main left bronchus was detected; the bronchial biopsy confirmed the CIS. The CIS was treated twice with endobronchial argon plasma coagulation. Due to local recurrence, the patient finally underwent pneumonectomy.

Discussion

Since the development of transbronchial needle aspiration (TBNA) for flexible bronchoscopy for lung cancer staging in the eighties, false-positives (FP) have been rarely reported. A case is considered as FP when tumor cells are identified on transbronchial lymph node aspiration but tumor metastases are not found in nodes obtained by thoracotomy or mediastinoscopy. Probably the exceptionality of FP in TBNA sampling is an accurate estimation but not all the studies confirmed positive TBNA results with further invasive procedures. When the technique was first described [1] it was recommended to perform the TBNA prior to any manipulation in order to minimize the risk of contamination of the aspiration specimen and then avoid potential false-positive results.

Cropp [2] and cols were the first investigators to describe a false positive in a TBNA sampling. These authors postulated that tumor cells exfoliated from bronchogenic carcinoma could be located on the

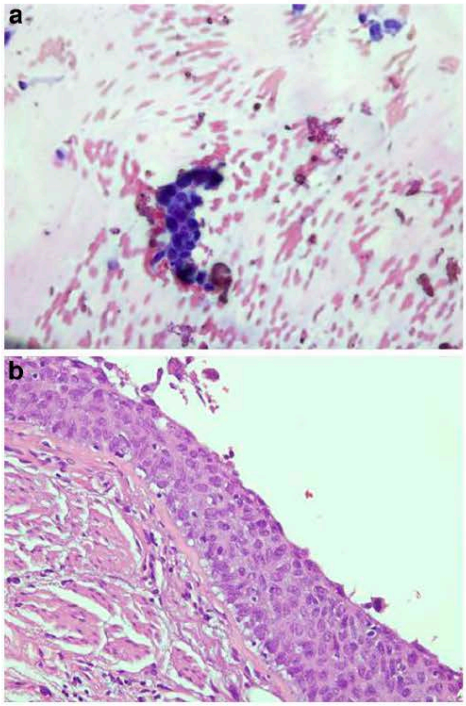

Figure 2 a: Transbronchial lymph node aspiration specimen: cluster of neoplastic squamous cells (no lymphocytes in this field) (Papanicolau stain, x400) b: Surgical specimen: carcinoma in situ (CIS) on the bronchial resection margin (Haematoxylin&Eosin stain, x400).

mucosa surface and trapped as the needle penetrated the tracheal wall and could therefore be collected during aspiration. Other authors described a FP case probably secondary to tumor sampling instead of lymph node [3]. For this reason, special care was recommended when an aspiration harboured neoplastic cells but no lymphocytes, especially in patients with minor radiological suspicion. Other cases of FP TBNA were attributed to needle contamination but overall TBNA has been considered highly reliable and FP have been considered clinically non-significant [4].

With the emergence of echo-probes for radial EBUS the accuracy of TBNA procedures increased and only one case of FP was registered in a series of Okamoto et al. [5] Since the introduction of echobronchoscopes, that provide real time assistance to TBNA no false-positive transbronchial lymph node aspirates have been described in any series, and consequently a specificity of 100 % has been conferred to this technique. Although, as previously, only a few studies performed with EBUS-rt-TBNA confirmed positive results with mediastinoscopy or thoracotomy.

EBUS allows real time guidance of the puncture avoiding incidental sampling of masses. The needle used for EBUS-rt-TBNA incorporates a stylet in the inner channel that prevents contamination. The only potential scenario for FP results of EBUS-rt-TBNA is the use of a single needle to sample more than one node after a positive result.

To our knowledge, the present case is the first FP documented for EBUS-rt-TBNA and is the first attributed to needle contamination by neoplastic cells from a bronchial CIS on the puncture point. As a mechanism similar to that suggested by Cropp and cols, probably malignant squamous cells from the CIS were introduced during the puncture and then aspirated. During the procedure the syringe suction was always attached and released just when the needle was inside the lymph node. Consequently, we do not consider incidental suction of the bronchial wall as a possible explanation for this case. Fortunately, in our patient the FP had no consequences on the staging that could change the choice of treatment. CIS are frequent in smoking patients with lung neoplasm, may be multicentric and difficult to identify with white light bronchoscopy [6].

Conclusions

The present case represents a fortuitous coincidence that might be considered when obtained results are contradictory in patients with squamous lung carcinoma. EBUS-rt-TBNA is a highly specific technique with a very low risk of contamination if minimal requirements are met.

Consent

Written informed consent was obtained from the patient for publication of this Case report and any accompanying images. A copy of the written consent is available for review by the Editor-in-Chief of this journal.

Competing interests

The authors claim no conflict of interest to declare.

Authors' contribution

JS-S: performed EBUS and wrote the manuscript. FA: performed EBUS. PS: performed autofluorescence bronchoscopy and attended the patient. ML: performed pathological examination. EC: performed pathological examination. JA: perfomed surgery on the patient. EM: performed EBUS and revised the manuscript. JR-M: revised the manuscript. All authors read and approved the final manuscript.

Financial support

This paper has been published with funds from a grant from the Spanish Government (FIS 09/01612).

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Received: 15 November 2011 **Accepted:** 7 August 2012
Published: 14 August 2012

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doi:10.1186/1749-8090-7-74
Cite this article as: Sanz-Santos *et al.*: **False positive endobronchial ultrasound-guided real-time transbronchial needle aspiration secondary to bronchial carcinoma in situ at the point of puncture: a case report.** *Journal of Cardiothoracic Surgery* 2012 **7**:74.

Clin Exp Metastasis (2013) 30:521–528
DOI 10.1007/s10585-012-9556-3

RESEARCH PAPER

Endobronchial ultrasound-guided transbronchial needle aspiration in the diagnosis of intrathoracic lymph node metastases from extrathoracic malignancies

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Received: 1 June 2012 / Accepted: 15 November 2012 / Published online: 30 November 2012

Abstract Intrathoracic lymph node enlargement is a common finding in patients with extrathoracic malignancies. Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a technique that is commonly used for lung cancer diagnosis and staging but that has not been widely investigated for the diagnosis of enlarged mediastinal and lobar lymph nodes in patients with extrathoracic malignancies. We conducted a retrospective study of 117 patients with extrathoracic malignancies who underwent EBUS-TBNA for diagnosis of intrathoracic lymph node enlargement from October 2005 to December 2009 and compared the EBUS-TBNA findings with the final diagnoses. EBUS-TBNA diagnosed mediastinal metastases in 51 of the 117 (43.6 %) cases and gave an alternate diagnosis or ruled out the presence of malignancy in 35 (56.4 %). Fourteen of these 35 patients underwent further surgical investigation, while the remaining 21 had clinical and radiological follow-up for 18 months. No false negatives were found in the surgery group. In the follow-up group, 13 patients had stable or regressive lymphadenopathy, and eight developed clinicoradiological progression and were assumed to have been false negatives by EBUS-TBNA. The sensitivity and negative predictive value of EBUS-TBNA were 86.4 and 75 %, respectively. Immunohistochemical staining (IHC) was performed in 80.4 % of the samples obtained by EBUS-TBNA. In samples obtained from ten patients with metastatic breast cancer, estrogen receptor expression was successfully assessed in eight patients and progesterone receptor and human epidermal growth factor receptor 2 in four. EBUS-TBNA is an accurate procedure for the diagnosis of thoracic lymph node metastases in patients with extrathoracic malignancies and should be an initial diagnostic tool in these patients. Furthermore, EBUS-TBNA can obtain high-quality specimens from metastatic lymph nodes for use in molecular analyses.

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Keywords Endobronchial ultrasound · Transbronchial needle aspiration · EBUS-TBNA · Mediastinal lymph node metastases · Extrathoracic malignancy · Immunohistochemistry

Introduction

Intrathoracic hilar and/or mediastinal nodal enlargement in patients with concurrent or previously diagnosed extrathoracic malignancies is a common finding both by computed tomography (CT) or positron emission tomography-computed tomography (PET-CT). Nodal enlargement can be found at the time of the initial diagnosis, which may affect staging and therefore be crucial for the choice of treatment, or it may be identified during or after the course of a radical treatment, which may imply a disease relapse. In addition, nodal enlargement may be from a primary intrathoracic malignancy, such as lung cancer, which will require a change in treatment. In all these situations, intrathoracic nodal enlargement requires pathologic confirmation and generally represents a challenge for the clinician.

For many years, mediastinoscopy was the only diagnostic procedure for mediastinal lymphadenopathy. However, this surgical technique requires general anesthesia and is not the most suitable for patients undergoing chemotherapy. Over the last years, endoscopy ultrasound-guided fine needle aspiration (EUS-FNA) has proven to be an alternative to surgery for sampling mediastinal nodes in these patients [1, 2], but it cannot reach all the nodal stations.

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a relatively novel technique whose usefulness in the diagnosis and staging of lung cancer [3] is widely recognized. However, few studies have focused on the use of EBUS-TBNA in the diagnosis of intrathoracic lymph node metastases in patients with extrathoracic malignancies [4–7]. Furthermore, although EBUS-TBNA has been used successfully to collect samples for molecular analyses in lung cancer patients [8], only one single study has reported the feasibility of EBUS-TBNA to obtain samples from metastatic extrathoracic malignancies that allow the performance of molecular analyses [9].

We have retrospectively assessed the value of EBUS-TBNA for the diagnosis of mediastinal lymph node metastases in patients with extrathoracic malignancies. In addition, we have examined the feasibility of obtaining sufficient high-quality tissue samples with EBUS-TBNA for ancillary molecular analyses which can provide additional diagnostic information for these patients.

Patients and methods

Patients

We retrospectively analyzed the clinical files of all patients with a concurrent or a previously diagnosed extrathoracic malignancy who were referred to our institution for EBUS-TBNA because of suspected intrathoracic nodal metastases from October 2005 to December 2009. The clinical suspicion of metastases was based on nodal enlargement (short axis > 10 mm) on CT (with or without lung lesions) [10] and/or on 2-fluoro-2-deoxy-D-glucose (FDG) uptake on PET-CT in all cases. Patients with a previous or concurrent pathologic diagnosis of intrathoracic malignancy previously to the performance of EBUS-TBNA were not included in the study.

Procedures

EBUS-TBNA was performed in an outpatient setting using a flexible bronchoscope (BF-UC160F-OL8, Olympus Optical Co Ltd., Tokyo, Japan) with a distal probe capable of producing linear parallel scans of the mediastinal and peribronchial tissues and a working channel suited to the performance of TBNA under direct ultrasound guidance. Local anesthesia and conscious sedation were achieved using topical lidocaine spray and intravenous midazolam, respectively [11]. Mediastinal and lobar nodes with a short-axis diameter of ≥5 mm identified during the procedure were sampled under direct ultrasound visualization with a 22-gauge cytology needle specially designed for EBUS-TBNA (NA-201SX-4022, Olympus Optical Co Ltd.).

The aspirates were recovered and placed on slides, fixed with 95 % ethanol and stained with haematoxylin for rapid on-site evaluation by a cytopathologist. An immediate assessment was given after each pass. Nodes were classified as "normal tissue negative for malignancy" when the sample contained 40 lymphocytes per high-power field in cellular areas of the smear and/or clusters of pigmented macrophages and contained no neoplastic cells or as "metastatic" when recognizable groups of malignant cells were present [12]. After this immediate assessment, Papanicolaou staining was completed in the laboratory. Whenever the cytopathologist considered it to be necessary, additional material was obtained and processed as cell blocks for ancillary studies. Cell blocks were prepared by air-drying the slides to clot and scraping them into 10 % formalin for subsequent processing in the laboratory. Blocks were embedded in paraffin and sectioned (5 μm thickness). Routine haematoxylin–eosin staining was used on cell-block sections, and IHC was used whenever this was needed for tumor origin identification.

In patients with metastatic breast cancer the expression of estrogen receptor (ER), progesterone receptor (pgR) and

human epidermal growth factor receptor 2 (HER2) was analyzed in EBUS-TBNA recovered material. ER and PgR were evaluated using IHC with the anti-estrogen receptor antibody 6F11 and the anti-progesterone receptor antibody 5D10 respectively (Novocastra, Newcastle Upon Tyne, England). The threshold values for reporting positivity were 1 % of tumor cells [13]. For HER2, staining was performed using Herceptest (DakoCytomation, Carpinteria, CA, USA), and positive cases were confirmed by means of fluorescence in situ hybridization (FISH; Vysis PathVysion, Downers Grove, IL, USA).

When EBUS-TBNA findings were positive for malignancy, they were assumed to be true positives and no further tissue confirmation was requested. Patients in whom EBUS-TBNA did not unequivocally show the presence of malignancy or an alternate benign diagnosis were referred for additional investigations including mediastinoscopy or thoracotomy to obtain a reference pathology result. Clinical and radiological follow-up for at least 18 months was used if the clinician judged this was sufficient. No patients were lost to follow-up.

Statistical analysis

Data were analyzed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA). Results were expressed as absolute and relative frequencies for categorical variables and as means and standard deviations (SD) or as medians and interquartile ranges (IQR), for continuous variables. Specificity and positive predictive value were assumed to be 100 %. Sensitivity (number of true positives/number of true positives + number of false negatives), negative predictive value (NPV; number of true negatives/number of true negatives + number of false negatives) and accuracy (number of true positives + number of true negatives/number of true positives + number of false positives + number of true negatives + number of false negatives) were calculated for the diagnosis of intrathoracic nodal metastases from extrathoracic malignancies.

Results

One hundred and seventeen patients were reviewed. Table 1 shows the patient characteristics. Head and neck, colorectal, breast and prostate carcinoma represented 60 % of the malignancies included. Figure 1 shows the disposition of patients by EBUS-TBNA findings and follow-up.

EBUS-TBNA identified intrathoracic nodal metastases from an extrathoracic malignancy in 51 (43.5 %) cases, a primary intrathoracic malignancy with lymph node involvement in 27 (23 %) cases (26 lung cancer, 1 lymphoma), and an alternative benign diagnosis in four (3.4 %) cases (2 sarcoidosis, 2 sarcoid-like reaction—non-caseating granulomatous inflammation, clinically inconsistent with sarcoidosis, with fungal and mycobacterial negative cultures) (Fig. 1). For all these patients, no histological confirmation was requested. In the two patients with sarcoid-like reaction, clinical and radiological follow-up was carried out to confirm or rule out a possible tumor as the cause [14]. After 6 months, one of these two patients developed a lymphoma.

EBUS-TBNA found normal lymph node tissue in 35 (29.9 %) patients, 14 (11.9 %) of whom underwent surgery to obtain a reference pathology result. Seven (6 %) of these patients had lung cancer without nodal involvement, four (3.4 %) had a lung metastasis from an extrathoracic malignancy without nodal involvement, and in the remaining three patients an alternative benign diagnosis was established (one case of silicosis and two cases of nodal tuberculosis). These three cases that have a diagnosis of normal lymph node tissue established with EBUS-TBNA but an alternative benign disease after surgery, although considered as true negatives

Table 1 Patient characteristics ($N = 117$)

Characteristic	N (%)
Age (years), mean(SD)	65.3 (12.3)
Gender (male), n (%)	77 (66)
Extrathoracic malignancy n (%)	
Head and neck carcinoma	21 (18)
Colorectal carcinoma	19 (16.4)
Breast carcinoma	18 (15.4)
Prostate carcinoma	12 (10.2)
Urothelial carcinoma	9 (7.7)
Renal carcinoma	7 (6)
Bladder carcinoma	5 (4.3)
Sarcoma	5 (4.3)
Stomach carcinoma	4 (3.5)
Unknown origin carcinoma	4 (3.4)
Melanoma	3 (2.5)
Endometrial carcinoma	2 (1.7)
Thyroid carcinoma	2 (1.7)
Other*	6 (4.8)
Extrathoracic malignancy status	
Previously diagnosed	65 (55.5)
Concurrent	52 (44.1)
During diagnosis	16 (13.7)
During treatment	36 (30.8)
CT findings	
Intrathoracic nodal enlargement without lung lesion	46 (39.3)
Intrathoracic nodal enlargement with solitary nodule/mass	44 (37.6)
Intrathoracic nodal enlargement with multiple nodules/masses	27 (23.1)

* cervical, ovarian, pancreas, adrenal, testis, extrathoracic lymphoma

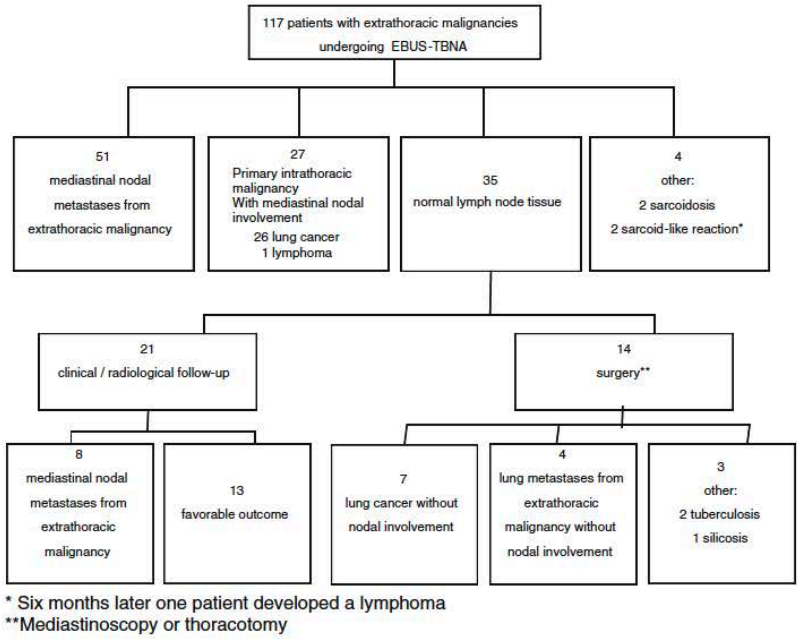

Fig. 1 Distribution of patients by EBUS-TBNA findings, follow-up and final diagnosis

for malignancy, were not included in the analysis of NPV. No false negatives were found among the 14 patients who underwent surgery (Fig. 1).

The remaining 21 (17.9 %) patients with neither an unequivocal diagnosis of metastases nor an alternate diagnosis underwent clinical and radiological follow-up for at least 18 months. In 13 (11.1 %) of these patients, stable or regressive lymphadenopathy, consistent with a benign diagnosis, was confirmed. In eight (6.8 %) of these patients, radiological follow-up found mediastinal and/or pulmonary progression, and the EBUS-TBNA findings were categorized as false negatives (Fig. 1). Most of these patients had thoracic progression, including nodal enlargement and/or multiple pulmonary nodes or masses, or extrathoracic metastases, including abdominal or intracranial metastases, and due to ethical reasons or because the patients rejected surgery, no histological confirmation was requested. Table 2 shows the final diagnoses for all 117 patients.

The sensitivity, negative predictive value (NPV) and accuracy of EBUS-TBNA for the diagnosis of nodal metastases from extrathoracic malignancies were 86.4 % (51/51 + 8), 75 % (24/24 + 8) and 90.3 % (51 + 24/51 + 0 + 24 + 8) respectively. No complications appeared during the EBUS-TBNA procedure or in the 2 weeks following the procedure.

Table 2 Final diagnosis of 117 patients with extrathoracic malignancy undergoing EBUS-TBNA for diagnosis of intrathoracic lymph node enlargement, *n* (%)

Mediastinal nodal metastases from extrathoracic malignancy	59 (50.4)
Pulmonary metastases from extrathoracic malignancy without nodal involvement	4 (3.4)
Intrathoracic malignancy	
Lung cancer	33 (28.2)
Lymphoma	1 (0.8)
Benign lymph node disease	
Normal lymph node tissue	13 (11.1)
Sarcoidosis	2 (1.7)
Tuberculosis	2 (1.7)
Sarcoid-like reaction	2 (1.7)
Silicosis	1 (0.8)

Among the 51 patients in whom EBUS-TBNA found lymph node metastases (Table 3), colorectal and breast carcinoma were the most frequent primary malignancies (10 [19.6 %] patients each). Thirty-one (60.7 %) patients showed nodal enlargement on EBUS, and 91 % of the patients having a PET-CT showed high FDG avidity. Half of the patients had extrathoracic spread. Twenty-nine patients (56.8 %) had a median disease-free status of

Table 3 Characteristics of patients with intrathoracic nodal metastases from extrathoracic malignancies diagnosed by EBUS-TBNA ($N = 51$)

Characteristic	*N* (%)
Age (years), m (SD)	62.2 (13.2)
Gender (males)	27 (53)
Intra/extrathoracic spread	24/27
Malignancy	
Colorectal carcinoma	10
Breast carcinoma	10
Unknown origin carcinoma	4
Renal carcinoma	4
Stomach carcinoma	3
Head and neck carcinoma	3
Prostate carcinoma	2
Thyroid carcinoma	2
Urothelial carcinoma	2
Melanoma	2
Sarcoma	2
Others	7
Extrathoracic malignancy status	
Previously diagnosed	29 (56.9)
Concurrent	22 (43.1)
During diagnosis	17 (33.3)
During treatment	5 (9.8)
Location of malignant nodes	
Lobar	11 (21.6)
Mediastinal	40 (78.4)
Upper right paratracheal	1 (2)
Subcarinal	19 (37.3)
Lower left paratracheal	3 (5.9)
Lower right paratracheal	17 (33.3)
Characteristics of malignant nodes	
FDG avidity**	9.5 ± 4.6
Size (short-axis diameter)***	11.9 (8.4–8)

* one case each of bladder, cervical, endometrial, testis, ovarian, pancreatic and adrenal cancer

** In Standardized Uptake Value (SUV) (SD)

*** In mm (range)

58 months (IQR, 45–112) from diagnosis of the primary malignancy. Five had had a previous pulmonary metastasectomy, with a mean time of 29 months from surgery. Eleven (21.6 %) patients presented with a single mass or nodule on CT scans and could thus have been candidates for metastasectomy, but surgery was ruled out based on the EBUS-TBNA findings.

Cell blocks were obtained from 47 (92 %) of the 51 patients with lymph node metastases diagnosed by EBUS, and IHC was performed in 41 (80.5 %) (Figs. 2, 3). IHC was not considered necessary for the diagnosis in three (5.8 %) patients who had had pharyngeal or laryngeal

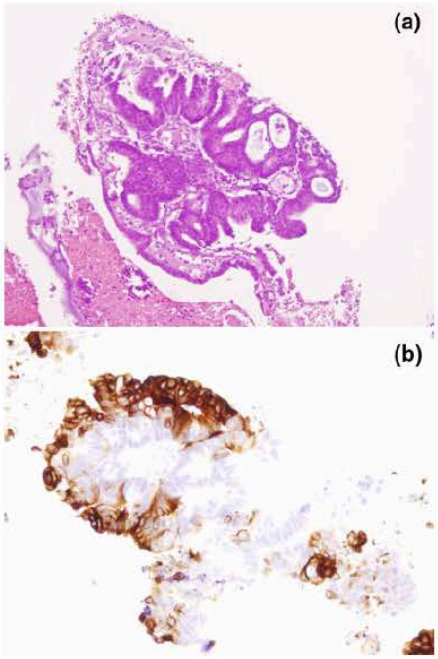

Fig. 2 **a** Cell block (haematoxylin–eosin 20×): mediastinal lymph node metastasis from colon adenocarcinoma. **b** At the immunohistochemical analysis, tumor cells were positive for keratin 20 (20×). Immunohistochemistry was consistent with metastasis from colon cancer

squamous cell carcinoma and seven (13.7 %) additional patients with different malignancies since the recovered material was morphologically consistent with the previously diagnosed extrathoracic malignancy.

In the ten patients with metastatic breast carcinoma, ER expression was assessed in eight cases and PgR and HER2 in four. PgR status had changed in only one patient, while ER and HER2 status showed no change.

Discussion

Our study confirms that EBUS-TBNA can diagnose thoracic lymph node metastases from extrathoracic malignancies with sensitivity and NPV similar to those of the two previous major studies [4, 5] and to findings in lung cancer staging [3]. Moreover, in our cohort of 117 patients, implementation of EBUS-TBNA obviated invasive surgical diagnostic procedures in nearly three quarters of the patients and allowed IHC and additional molecular analysis to be performed in more than 80 % of the cases.

In recent years, the development of novel therapies in cancer has led to an improvement in overall survival time

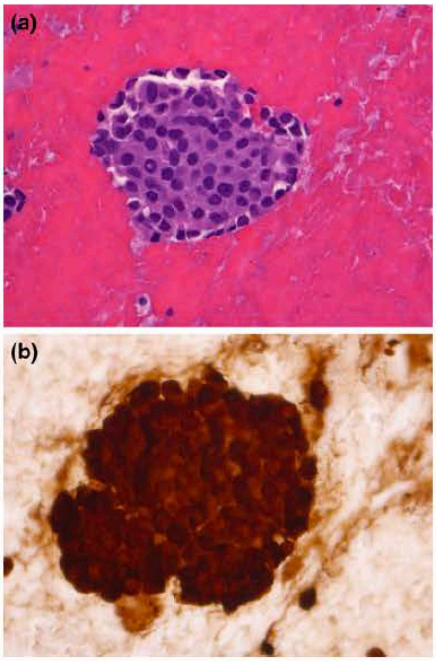

Fig. 3 **a** Cell block (haematoxylin–eosin 40×): mediastinal lymph node metastasis from medullary thyroid carcinoma. **b** At the immunohistochemical analysis, tumor cells were positive for calcitonin (40×). Immunohistochemistry was consistent with metastasis from medullary thryroid carcinoma

[15]. Cancer patients are now given periodic medical controls, and the detection of thoracic lymphadenopathy in patients with extrathoracic malignancies is no longer uncommon [16]. This progress has been accompanied by a parallel advance in minimally invasive techniques, such as EUS-FNA and EBUS-TBNA, which have proven to be a viable alternative to mediastinoscopy. However, mediastinoscopy requires general anesthesia, increasing both costs and patient risk [17, 18]. Several studies [1, 2] have evaluated the use EUS-FNA as a substitute for mediastinoscopy. In a retrospective series of 75 patients, EUS-FNA had a sensitivity of 86 % and NPV of 72 % [1], and in a short prospective study of 20 patients, the sensitivity of EUS-FNA was 68 % [2]. However, compared to EUS-FNA, EBUS-TBNA has the advantage of its bilateral hilar and mediastinal reach while EUS-FNA reaches only the left paratracheal, aorto-pulmonary window, subcarinal and paraesophageal mediastinal lymph node stations. In our series, ten (21.6 %) hilar and 18 (35.3 %) right paratracheal metastatic nodes would have been inaccessible by EUS-FNA.

EBUS-TBNA has long been used for diagnosing and staging lung cancer, but now there is a wealth of evidence to suggest that it has other uses [19]. Several studies have focused on the use of EBUS-TBNA for the diagnosis of sarcoidosis [20], but few have used it specifically for the detection of thoracic nodal metastases from extrathoracic malignancies [4–7]. In a retrospective series of 92 patients, EBUS-TBNA had a sensitivity of 85 % and a NPV of 76 % [5], and a similar study of 161 patients reported a sensitivity of 87 % and a NPV of 73 % [4]. In the present study, sensitivity and NPV were 86.4 and 75 %, respectively, which is similar to these two previous studies [4, 5]. The NPV of 75 % reported in this and in previous studies, however, justifies the referral of patients with neither an unequivocal diagnosis of malignancy nor an alternative diagnosis for additional tests, which may include mediastinoscopy.

Of the 44 patients with a single node mass or nodule on CT scans who were candidates for surgery, intrathoracic lymph node metastases from an extrathoracic malignancy were detected by EBUS in 11 (25 %), and surgery was ruled out for these patients based on the EBUS-TBNA findings. As the likelihood of a curative treatment after a metastasectomy depends on lymph node involvement, the European Society of Thoracic Surgeons recommends excluding patients with lymph node metastases from pulmonary metastasectomy with intent to cure. The incidence of lymph node involvement in patients undergoing pulmonary metastasectomy is estimated to be around 20 % [21]. The use of mediastinoscopy for selecting patients for surgery is unusual [22], and only one study [23] has examined the usefulness of mediastinoscopy in patients with lung metastases eligible for surgery; lymph node metastases were found in 10 % of the patients. In this clinical setting, EBUS-TBNA is thus a preferable approach, and the present study demonstrates the capabilities of EBUS-TBNA for the accurate selection of patients for therapeutic metastasectomy, as had been suggested by preliminary studies [24].

The present study has confirmed the ability of EBUS-TBNA to obtain high-quality specimens for ancillary studies, such as IHC and molecular analyses. In fact, IHC was performed in 80.4 % of the 51 patients diagnosed with mediastinal lymph node metastases. Molecular analysis is commonly used in samples obtained by EBUS-TBNA from patients with lung cancer, where they have been used to distinguish different types of non-small-cell lung cancer [25, 26] and to detect epidermal growth factor mutations [27] and the EML4-ALK fusion gene [8]. In the present study, among ten patients with metastatic breast cancer, it was possible to analyze ER expression in eight patients and PgR and HER2 expression in four. Since ER, PgR and HER2 expression levels can change in metastatic lesions in patients with breast cancer [28] and lead to drug resistance, a biopsy of suspected metastatic lesions is recommended in these patients for the analysis of ER, PgR and HER2 expression [29, 30]. The present study confirms that EBUS-

TBNA is a feasible minimally invasive method for obtaining samples for these molecular analyses.

There are a number of limitations in this study. Firstly, the fact that it is a retrospective study implies a certain selection bias. Secondly, no histological confirmation was obtained for cases diagnosed as negative by EBUS-TBNA, including those cases identified as false negatives during clinical and radiological follow-up. A prospective study with well-defined criteria for inclusion and histological confirmation could overcome both these limitations.

In conclusion, EBUS-TBNA is a simple, safe and accurate procedure for the diagnosis of thoracic lymph node metastases in patients with a concurrent or previously diagnosed extrathoracic malignancy. Our findings lead us to recommend the use of EBUS-TBNA as an initial diagnostic technique in these patients. Furthermore, EBUS-TBNA can obtain high quality specimens from metastatic lymph nodes for use in molecular analyses.

Acknowledgments The authors would like to thank Ms Renée Grupp Meissner for her assistance in drafting the manuscript.

Grant support Funded partially by FUCAP (Catalan Foundation for Pulmonology), FIS 0901612 and Ciberes-Ciber de Enfermedades Respiratorias.

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Ultrasound in Med. & Biol., Vol. 38, No. 7, pp. 1163–1168, 2012

0301-5629/$ - see front matter

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doi:10.1016/j.ultrasmedbio.2012.03.012

● *Original Contribution*

INTRA- AND INTEROBSERVER AGREEMENT AMONG BRONCHIAL ENDOSONOGRAPHERS FOR THE DESCRIPTION OF INTRATHORACIC LYMPH NODES

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(*Received* 7 *December* 2011; *revised* 28 *February* 2012; *in final form* 14 *March* 2012)

Abstract—Several sonographic features observed by endobronchial ultrasonography have been suggested to be useful to predict malignancy in mediastinal lymph nodes. To evaluate agreement to describe sonographic features, 28 video images were evaluated twice by eight expert bronchoscopists. The observers reviewed each case for the presence of coagulation necrosis sign (CNS), central hilar structure (CHS), heterogeneity, distinct margin, round shape, size >1 cm and malignancy. Intraobserver agreement was almost perfect for size ($\kappa = 0.826$), substantial for CNS ($\kappa = 0.721$) and shape ($\kappa = 0.615$), and moderate for CHS ($\kappa = 0.565$), heterogeneity ($\kappa = 0.441$) and margin ($\kappa = 0.407$). Interobserver agreement was substantial for size ($\kappa = 0.641$), moderate for shape ($\kappa = 0.445$), and fair for CNS ($\kappa = 0.340$) and margin ($\kappa = 0.274$). In conclusion, inter- and intraobserver agreement of the endosonographic features for mediastinal or hilar lymph nodes is good for shape or size but not good enough for the other ultrasonographic features. (E-mail: ignasi.g.olive@gmail.com)

Key Words: **Agreement, EBUS, Interobserver agreement, Intraobserver agreement, Observer dependency, Sonographic features.**

INTRODUCTION

Endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) is a minimally invasive technique that has shown to be useful in mediastinal staging of lung cancer (Herth et al. 2006). Although it has been suggested that some sonographic features such as nodal size or shape as detected by EBUS could be used as predictors of malignancy (Garcia-Olivé et al. 2009; Wang Memoli et al. 2011), there has only been one attempt to create an EBUS imaging classification system (Fujiwara et al. 2010). Sonographic features should not substitute biopsies, as studies have shown that endoscopic ultrasound fine-needle aspiration (EUS-FNA) is superior to imaging by EUS alone (Chen et al. 2004) but these features could be useful to increase EBUS-TBNA negative predictive value (Fujiwara et al. 2010). Nevertheless, the degree to which endobronchial endosonographers agree on these nodal sonographic features remains unclear. The aim of this study was to evaluate the degree of agreement among endosonographers for the description of sonographic features of mediastinal lymph nodes.

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METHODS

Patients

Digital video images of mediastinal lymph nodes visualized by EBUS from 21 consecutive patients were prospectively recorded from January 2011 to February 2011. All these patients had lung cancer or suspected lung cancer and none had received previous treatment. All patients agreed to participate and the research protocol was approved by the regional ethics committee (Ethics Committee for Clinical Research of the Hospital Germans Trias i Pujol, registration number EO-11-099).

EBUS-TBNA

EBUS was performed under local anaesthesia and sedation, using a flexible bronchoscope (BF-UC180F-OL8; Olympus Optical Co., Tokyo, Japan) with a distal probe capable of producing linear parallel scans of the mediastinal and parabronchial tissues and a working channel suited to the performance of TBNA under direct ultrasound guidance. The ultrasound features were processed in a dedicated ultrasound processor (EU-ME1; Olympus Optical Co.). Local anaesthesia and sedation were administered using topical lidocaine spray and intravenous propofol, respectively, in accordance with standard recommendations (British Thoracic Society 2001; Reed 1992). Nodes with a short axis diameter of ≥5 mm that were detected during the procedure were sampled under direct ultrasound visualization with a 22-gauge cytology needle specially designed for EBUS-TBNA (NA2015X-4022; Olympus Optical Co.). Negative pressure was maintained at the proximal end of the catheter while the needle was pushed forward and backward inside the node. The needle was removed after sampling, rapid on-site cytologic examination was performed and the remaining material was processed as a histology core. Pathology findings were classified as being extracted from a normal node when it contained predominantly lymphocytic cells and no neoplastic cells, or from a metastatic node when recognizable groups of neoplastic cells were found.

Aspirates containing bronchial or blood cells alone were considered unsatisfactory; in these situations, the procedure was repeated up to three times.

All EBUS examinations were performed by two experienced endosonographers from the Respiratory Department at the Hospital Universitari Germans Trias i Pujol of Badalona (Barcelona), Spain.

Digital video images

Digital video images were uploaded on a web page, one for each lymph node. Eight endobronchial endosonographers from seven different institutions with at least 3 years of experience with EBUS, blinded to the results of EBUS-TBNA, were given a username and password and asked to review all these videos. In the web page they had to choose between yes or no, for each node sonographic feature proposed by Fujiwara et al. (2010), as follows: (1) short-axis size of more than 1 cm, (2) round, (3) distinct margin, (4) homogeneous, (5) central hilar structure (CHS) presence and (6) coagulation necrosis sign (CNS) presence (Fig. 1). They were also asked to give their subjective diagnosis as to whether the node was malignant or not. One month after the last reviewer had completed the questionnaire, all the reviewers were asked to answer it again, to evaluate intraobserver agreement.

The average duration of video images was 30 s and reviewers could watch each video image as many times as necessary. It was possible to freeze the video at any point and replay video images. Each video had a few seconds of duration with Doppler images.

Statistical analysis

The degree of agreement between raters was assessed by means of Cohen's kappa (κ), which improves upon simple percentage of agreement by taking into account the agreement occurring by chance (Landis and Koch 1977). Kappa values range from 0 (when there is no agreement other than what would be expected by chance) to 1 (when the agreement is perfect), though negative values are also possible in the case of absence of agreement. For this study, κ values greater then 0.81 were considered to be almost in perfect agreement; 0.61–0.80 were considered substantial; 0.41–0.60, moderate; 0.21–0.40, fair; and 0.00–0.20 were considered as slight agreement (Landis and Koch 1977).

We calculated two kappas for each sign of malignancy, namely the mean *intra*-rater κ and the mean *inter*-rater κ. The *intra*-rater κ of a rater was calculated as the agreement between the two ratings conducted by the rater. Ninety-five percent confidence intervals of the

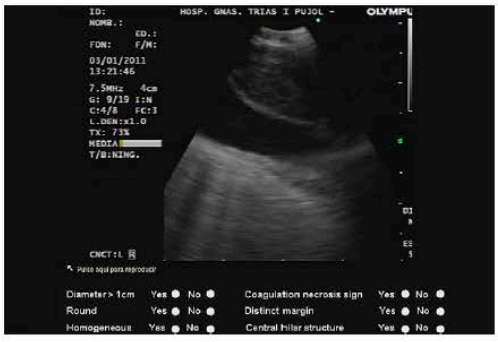

Fig. 1. Questionnaire web page screenshot.

Table 1. Characteristics of lymph nodes sampled

Node	Location	Cytological diagnosis
1	4R	Non-necrotizing granuloma
2	4R	Normal node
3	4L	Normal node
4	2L	Adenocarcinoma
5	7	NSCLC
6	4R	Adenocarcinoma
7	4R	Normal node
8	12R	Normal node
9	7	Poorly differentiated carcinoma
10	10R	Antracosis
11	7	Adenocarcinoma
12	4R	SCLC
13	4R	Squamous-cell carcinoma
14	4R	Normal node
15	7	Normal node
17	11L	Normal node
18	11L	Normal node
19	4R	Normal node
20	7	Adenocarcinoma
21	4L	Normal node
22	7	Normal node
23	2R	NSCLC
24	4L	Normal node
25	4R	Normal node
26	7	Normal node
27	11L	Normal node
28	11L	Adenocarcinoma

NSCLC = non–small cell lung cancer; SCLC = small cell lung cancer.

mean intra-rater κ were derived by simple bootstrapping, *i.e.*, the distribution of the sample mean was found by repeated resampling with replacement of the original intra-rater kappas. The *inter*-rater κ of two raters was calculated as the mean agreement between the ratings of one rater and the ratings of the other rater. Here, the bootstrapping strategy restricted each rater's information to be used in only one κ, to ensure that elements in any resample were statistically independent.

All calculations were conducted in R (R Foundation for Statistical Computing 2008) using the kappa2 command combined with new bootstrapping scripts written for the purpose of this study.

RESULTS

The characteristics (location and cytologic diagnosis) of lymph nodes sampled by TBNA are shown in Table 1. Intra- and interobserver agreements for individual features of lymph nodes visualized by EBUS are shown in Table 2.

There was almost perfect intra-rater agreement for size, with a κ of 0.826 (95% confidence interval [CI] 0.729–0.908). CNS and shape had substantial intraobserver agreement, with a κ of 0.721 (95% CI 0.533–0.902) and 0.615 (95% CI 0.542–0.687), respectively. The lymph node's CHS absence, heterogeneity and distinct margin had a moderate intraobserver agreement, with a κ of 0.565 (95% CI 0.376–0.732), 0.441 (95% CI 0.272–0.614) and 0.407 (95% CI 0.264–0.533), respectively.

The lymph node's size had a substantial interobserver agreement with a κ of 0.641 (95% CI 0.452–0.823). Nodal shape had a moderate interobserver agreement with a κ of 0.445 (95% CI 0.338–0.563). The presence of CNS and margin had a fair interobserver agreement, with a κ of 0.340 (95% CI 0.052–0.649) and 0.274 (95% CI 0.108–0.416), respectively.

Results for malignant lymph nodes are shown in Table 3. There was an almost perfect intra-rater agreement for size, with a κ of 0.907 (95% CI 0.820–0.973). Intra-rater agreement for CHS was fair, with a κ of 0.213 (95% CI 0.013–0.424).

Interobserver agreement was substantial for size, with a κ of 0.754 (95% CI 0.526–0.934). On the contrary, there was no effective interobserver agreement for CHS and heterogeneity, with a κ of 0.208 (95% CI –0.030–0.438) and 0.080 (95% CI –0.183–0.380).

Results for nonmalignant lymph nodes are shown in Table 4. Intra-rater agreement was substantial for size, shape and CHS, with a κ of 0.774 (95% CI 0.642–0.895), 0.637 (95% CI 0.485–0.790) and 0.631 (95% CI 0.449–0.796), respectively. Shape and size had a moderate interobserver agreement, with a κ of 0.517 (95% CI 0.392–0.703) and 0.515 (95% CI 0.316–0.773), respectively.

Table 2. Mean intra- and inter-rater agreement

	Intra-rater agreement Mean kappa (κ)	95% CI	Inter-rater agreement Mean kappa (κ)	95% CI
Reviewer's diagnosis (malignant or not)	0.555	0.487, 0.627	0.337	0.175, 0.473
Proposed signs of malignancy				
CNS present	0.721	0.533, 0.902	0.340	0.052, 0.649
CHS absent	0.565	0.376, 0.732	0.251	−0.057*, 0.558
Heterogeneous echogenicity	0.441	0.272, 0.614	0.098	−0.073*, 0.332
Distinct margin	0.407	0.264, 0.533	0.274	0.108, 0.416
Round shape	0.615	0.542, 0.687	0.445	0.338, 0.563
Size >1 cm	0.826	0.729, 0.908	0.641	0.452, 0.823

CHS = central hilar structure; CNS = coagulation necrosis sign.
* Negative values of κ indicate absence of effective agreement.

Table 3. Mean intra- and inter-rater agreement (malignant nodes)

	Intra-rater agreement Mean kappa (κ)	95% CI	Inter-rater agreement Mean kappa (κ)	95% CI
Reviewer's diagnosis (malignant or not)	0.627	0.445, 0.809	0.423	0.170, 0.662
Proposed signs of malignancy				
CNS present	0.713	0.528, 0.886	0.451	0.125, 0.755
CHS absent	0.213	0.013, 0.424	0.208	−0.030*, 0.438
Heterogeneous echogenicity	0.502	0.258, 0.718	0.080	−0.183*, 0.380
Distinct margin	0.581	0.391, 0.732	0.385	0.172, 0.612
Round shape	0.565	0.403, 0.728	0.288	0.109, 0.529
Size >1 cm	0.907	0.820, 0.973	0.754	0.526, 0.934

CHS = central hilar structure; CNS = coagulation necrosis sign.
* Negative values of κ indicate absence of effective agreement.

There was no effective interobserver agreement for CNS, CHS and heterogeneity, with a κ of 0.025 (95% CI –0.121–0.472), 0.260 (95% CI –0.046–0.580) and 0.114 (95% CI –0.113–0.358), respectively.

Intra-rater agreement for the diagnosis of malignancy was moderate, with a κ of 0.555 (95% CI 0.487–0.627). Inter-rater agreement was fair, with a κ of 0.337 (95% CI 0.175–0.473).

DISCUSSION

The intraobserver agreement on the endosonographic features of lymph nodes was almost perfect for size, substantial for CNS and shape and moderate for CHS, heterogeneity and margin. Interobserver agreement was substantial for size, moderate for shape and fair for CNS and margin. In the overall subjective impression of benign vs. malignant lymph node, intraobserver agreement was moderate and inteobserver agreement was fair.

Although ultrasonography has proven to be a valuable tool for the detection of enlarged lymph nodes, differentiation between benign and malignant nodal disease remains a problem. Ultrasound probes enable differentiation between the central echogenic oval hilus and the peripheral concentric hypoechoic cortex of the lymph node. Changes in the shape of both components may suggest the presence of disease (benign or malignant), even in the presence of minimal node enlargement (Vassallo et al. 1992). Nevertheless, because the sonographic appearance of benign lymphadenopaties is similar to that of reactive malignant nodes, the diagnosis is still most often based on histology (Ahuja and Ying 2004).

To the best of our knowledge, this is the first study that evaluates intra- and interobserver agreement to describe ultrasonograhic features of lymph nodes visualized by EBUS. Nevertheless, there are several articles in the field of endoscopic ultrasonography (EUS), which have studied interobserver agreement, either for the diagnosis of pancreatic cystic lesions (Ahmad et al. 2003) or for the description of endosonographic features of lymph nodes in aerodigestive malignancies (de Melo et al. 2011). In this latter article, the authors studied interobserver agreement for echogenicity (hypoechoic vs. other), shape (round vs. other), border (sharp vs. fuzzy) and final diagnosis (malignant, benign or indeterminate). Agreement was fair for shape, moderate for echogenicity and border and substantial for the final diagnosis (malignant or benign).

Sonographic EBUS features of lymph nodes during EBUS-TBNA have shown to be helpful in the prediction of benign lymph nodes in lung cancer patients (Fujiwara

Table 4. Mean intra- and inter-rater agreement (nonmalignant nodes)

	Intra-rater agreement Mean kappa (κ)	95% CI	Inter-rater agreement Mean kappa (κ)	95% CI
Reviewer's diagnosis (malignant or not)	0.484	0.332, 0.648	0.243	0.074, 0.431
Proposed signs of malignancy				
CNS present	0.500	0.121, 0.862	0.025	−0.121*, 0.472
CHS absent	0.631	0.449, 0.796	0.260	−0.046*, 0.580
Heterogeneous echogenicity	0.439	0.242, 0.617	0.114	−0.113*, 0.358
Distinct margin	0.349	0.208, 0.470	0.212	0.062, 0.377
Round shape	0.637	0.485, 0.790	0.517	0.392, 0.703
Size >1 cm	0.774	0.642, 0.895	0.515	0.316, 0.773

CHS = central hilar structure; CNS = coagulation necrosis sign.
* Negative values of κ indicate absence of effective agreement.

et al. 2010) but should not rule out doing a biopsy of those nodes. Obtaining tissue diagnosis from suspicious lymph nodes should still be mandatory. However, the lack of lymph node EBUS malignant features could be reassuring in confirming the true negativity of that lymph node (Fujiwara et al. 2010), thus, increasing the negative predictive value of the technique. In fact, this situation has already been described in the field of conventional ultrasonography (US) for papillary thyroid cancer (PTC) (Kwak et al. 2008). In this study, Kwak et al. concluded that the probability of malignancy was much lower in thyroid nodules with benign US findings, even if the FNA biopsy was read as suspicious for PTC (Kwak et al. 2008).

Contrast enhanced ultrasound (CEU) could be useful for differentiating benign from malignant lymph nodes, according to the pattern of enhancement (Blomley et al. 2001; Aoiki et al. 2011). It has been used in endoscopic ultrasonography (Kanamori et al. 2006) but no study has reported its use in EBUS.

An alternative to these ultrasonographic features could be the use of new ultrasonography-associated technology, such as endosonographic elastography. This method uses mechanically induced deformations of structures in the B-mode image to quantify the elasticity of the tissue, which could allow the establishment of a classification as benign or malignant. There is no existing data about EBUS elastography, but it has been successfully used in EUS in the diagnosis of mediastinal lymph nodes (Janssen et al. 2007), with an excellent interobserver agreement ($\kappa = 0.84$).

Assessment of B-mode images from convex-type EBUS by artificial neural networks (ANN) has also been described (Tagaya et al. 2008). ANNs are computer-assisted support systems that mimic the biologic nervous system and are used in pattern recognition. In this case, diagnostic accuracy was significantly higher for ANNs than for expert surgeons (Tagaya et al. 2008).

Recently, Nguyen and colleagues found that gray-scale texture analysis of EBUS images could be useful to differentiate malignant and benign lymph nodes (Nguyen et al. 2012). Their preliminary results were comparable to fluorodeoxyglucose positron emission tomography and computed tomography (FDG-PET/CT) (Nguyen et al. 2012).

The main limitation of the study is that we used the results of the FNA to classify the lymph node as benign or malignant. Nevertheless, we have a 6-month follow-up that suggests that the benign nodes were not involved by micrometastasis. Another limitation of the study is that raters did not perform the technique by themselves but only had to review a previously recorded video. It seems reasonable to consider that agreement for size would have been lower if reviewers could have chosen their own ultrasound plane. On the other hand, and for the same reason, agreement for other features such as CHS or CNS might have been better.

The main strength of this study is that the images were obtained prospectively (with standard settings for both frequency and gain) and evaluated by bronchoscopists blinded to the cytologic result. Another strength is that images were not static captures but video images, which allowed us to investigate ultrasonographic features that required the use of Doppler.

In summary, the inter- and intraobserver agreement of the endosonographic features for mediastinal or hilar lymph nodes is good for shape or size, but not satisfactory for the other ultrasonographic features. Morphologic features of mediastinal lymph nodes could not substitute FNA but might be useful for targeting the most suitable nodes for FNA. New techniques are warranted to standardize the evaluation of lymph nodes by EBUS.

Acknowledgments—The authors thank A. Ruiz and A. Barrios for their technical assistance during the performance of the procedures. This work was supported by a grant from the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR); Catalan Society of Pulmonology (SOCAP); CibeRes–Ciber de Enfermedades Respiratorias; and Fondo de Investigación Sanitaria (FIS) (grant PS09/01612).

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Thoracic Cancer ISSN 1759-7706

INVITED REVIEW

The role of endobronchial ultrasound in central early lung cancer

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Keywords
early stage; endobronchial ultrasound; lung cancer.

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Received: 14 November 2011; accepted 12 December 2011.

doi: 10.1111/j.1759-7714.2011.00102.x

Abstract

Central early lung cancers (CELC) are tumors arising from the central airways, roentgenographically occult, which are usually diagnosed by bronchoscopy after a positive sputum cytology. Most CELCs are undetectable for conventional white light bronchoscopy (WLB) but can be identified under autofluorescence bronchoscopy (AFB). Although AFB increases the sensitivity of WLB in detecting CELC, its low specificity remains a problem. Surgery has been the most accepted treatment for CELCs; however 20–30% of patients suffering CELC tend to have multicentricities and usually present with poor cardiopulmonary status. Therefore, surgery is not suitable in most of the cases and other therapeutic options such as bronchoscopic treatments should be considered. Because most endoscopic treatments are unlikely to be curative if the tumor has spread beyond the bronchial cartilage, accurate evaluation of CELC bronchial wall invasion is critical before selecting a bronchoscopic treatment. Endobronchial ultrasound (EBUS) is a relatively new technique that has proven to be useful in the evaluation of the normal and cancer-invaded bronchial wall. Some authors have demonstrated that after adding EBUS assessment to AFB in autofluorescence-positive lesions the specificity increases from 50 to 90%. Other studies have focused on the ability of EBUS to detect bronchial wall invasion in patients with CELCs. They compared the EBUS images with pathological findings of surgical specimens of patients that underwent surgery; in most of the cases the correlation between EBUS and pathological findings increased over 90%. Furthermore, in patients not eligible for surgery, EBUS has proven to predict patients expected response to endoscopic treatments.

Introduction

Lung cancer is the leading cause of cancer deaths worldwide. Despite the new advances in treatment and diagnosis the overall 5-year survival rate is only 15%.[1] This poor survival is related to the fact that most lung cancers are detected at advanced non-surgical stages, when therapeutic options are limited. In this setting, early detection of lung cancer has become crucial. Recently several studies have focused on lung cancer screening by means of low-dose spiral computer tomography (CT). While many small peripheral lesions are detected on CT, centrally located early lung cancer (CELC) can remain roentgenographically occult and require bronchoscopic techniques for diagnosing. Over the last year new bronchoscopic imaging techniques including autofluorescence bronchoscopy (AFB) and narrow band imaging (NBI) have been developed; these new techniques have shown better sensitivity than conventional white light bronchoscopy (WLB) in CELC detection. Although AFB is a more sensitive process, low specificity remains a problem. Endobronchial ultrasound (EBUS) has proven to be an excellent method to visualize the multilayer structure of the bronchial wall and to predict the tracheobronchial wall invasion of intrathoracic malignancies. This article focuses on the role of EBUS in central early lung cancer detection.

Table 1 The Japan Lung Cancer criteria for central early lung cancer (CELC)

1) Location in subsegmental or more proximal bronchi or trachea.
2) The peripheral margin of the tumor visible bronchoscopically
3) The tumor size is less than 2 cm in greatest dimension
4) Squamous cell carcinoma is identified histologically

Central early stage lung cancer

The Japan Lung Cancer Society defined bronchoscopic (Table 1) and clinical criteria for CELC.[2] Basically CELCs are defined as squamous-cell carcinomas, with a tumor size less than 2 cm in greatest dimension, arising in the central airways, roentgeographically occult and without lymph node or distant metastases. According to the bronchoscopic features, CELCs can be classified into five categories: polypoid, nodular, thickened, invisible and mixed.[3]

CELCs are thought to be develop through multiple stages from squamous metaplasia to dysplasia, followed by carcinoma in situ (CIS), progressing to microinvasive and invasive tumors. Patients are suspected to have CELCs after positive sputum cytology, usually during lung cancer screening.

Endobronchial ultrasound (EBUS) and the bronchial wall

Currently there are two different systems of EBUS based on the location of the transducer: linear transducers incorporated at the tip of the bronchoscope (Olympus BF-UC180F, Olympus BF-UC160F, Pentax EB-1970UK), and echo probes (radial) with a rotating transducer that can be inserted through different ordinary flexible bronchoscopes. Linear transducers use low frequencies (5–7.5–10–12 Hz) and provide a sectorial view of the parabronchial structures. As lower frequencies give better penetration depth with less resolution, lineal EBUS are used basically for real-time transbronchial needle aspiration (TBNA) of hilar and mediastinal lesions. In contrast with linear transducers, probes with rotating transducers generate a complete circular image of the parabronchial structures and use higher frequencies (20–30 Hz). The standard frequency for a rotating transducer is 20 MHz which attains a resolution of 1 mm with a penetration depth of 4–5 cm. Depending on the size, radial probes can be used for locating peripheral nodes or masses (mini probes Olympus UM-S20-17S, Olympus UM-S30-20R) or for the visualization of the central airway wall and surrounding structures. The latter can incorporate the water-filled balloon (Olympus UM-BS20-26R) or may require the use of a balloon sheath (Olympus UM-S20-20R).

Ultrasonography of the central airway is usually performed with a flexible bronchoscope with a working channel over 2.8 mm in routine settings. Once the probe is placed in the airway lumen under visual control, the balloon is inflated until complete contact to the bronchial wall is accomplished, thereby obtaining a complete 360° view of the bronchial wall and the surrounding structures. While some authors[4] recommend general anesthesia with mechanical ventilation when complete occlusion of the airway is required (scanning of the trachea for instance), other authors affirm[5] that complete obstruction is well tolerated under local anesthesia and sedation for 20–30 seconds. In case of large tracheas of impaired lung function the balloon can be partially inflated while enhancing the contact to the bronchial wall with the bronchoscope.

The first study that described the endobronchial ultrasound appearance of the bronchial wall was published in 1992. Hürter and Hanrath performed EBUS using an ultrasound catheter originally designed for endovascular examinations. They described the bronchial wall on the EBUS as a three-layered structure.[6] In 1999 Kurimoto *et al.*[7] compared the endobronchial ultrasound images of normal and tumor invaded bronchi with the histological findings of surgical specimens in patients with lung cancer undergoing lobectomy. In the cartilaginous portion of trachea, extrapulmonary and intrapulmonary bronchi they characterized five layers: the first two correlated with the mucosa plus the balloon echo and submucosa respectively; the fourth hypoechoic layer corresponded to the cartilage, surrounded by two hyperechoic layers; and the third and fifth correlated with the perichondrium. In the membranous portion of the trachea and extrapulmonary bronchi, lacking cartilage, three ultrasonically distinct layers were imaged. With the development of more accurate probes other authors have been able to describe a sixth[8] and a seventh[9] layer beyond the cartilage (Table 2) corresponding to the supporting connective tissue and the adventitia respectively.

Since it was first described, EBUS has proven to be more accurate than CT to distinguish between tumor compression versus invasion of the bronchial wall in patients with lung cancer[10] and other thoracic malignancies.[11,12]Furthermore, EBUS has been rendered useful for the assessment of bronchial wall remodeling in patients with asthma; for measuring the thickness of the bronchial wall in lung transplantation rejection; as well as diagnosing central airway disorders such as tracheomalacia or relapsing polychondritis.[13–16]

Endobronchial ultrasound and central early lung cancer diagnosis

Most of CELCs present as subtle changes of the bronchial mucosa below the threshold of WLB. Thereby, the reported sensitivity of WLB to detect CELC is limited to 30%.[3] Autofluorescence bronchoscopy is a bronchoscopic technique based on the differences in the fluorescence properties of normal and malignant bronchial mucosa. AFB has shown to

Table 2 Echographic layers of the bronchial wall

Layer	Echo	Finding
Intra-pulmonary bronchus or cartilaginous portion of trachea or extra-pulmonary bronchi:		
First	Hyperechoid	Marginal echo (balloon and mucosa)
Second	Hypoechoid	Submucosa
Third	Hyperechoid	Inner side of the bronchial cartilage (perichondrium)
Fourth	Hypoechoid	Cartilage
Fifth	Hyperechoid	Outer side of the bronchial cartilage (perichondrium)
Sixth	Hypoechoid	Supporting connective tissue
Seventh	Hyperechoid	Adventitia
Membranous portion of trachea or extra-pulmonary bronchi:		
First	Hyperechoid	Marginal echo (balloon and mucosa)
Second	Hypoechoid	Smooth muscle
Third	Hyperechoid	Adventitia

improve the sensitivity for detection of CELC when used simultaneously with WLB[17] (Fig. 1). However, as many benign lesions such as inflammatory areas can be mistaken for CELC only by bronchoscopic inspection, bronchial biopsy still remains the gold standard. This low specificity in AFB can result in multiple unnecessary biopsies to a single patient, increasing the cost and risk of the procedure. Herth *et al.*[18] demonstrated that EBUS improves the classification of suspicious lesions detected by AFB. In a prospective study they recruited 332 patients with a high risk of lung cancer undergoing AFB and EBUS. AFB findings were classified as: class 1, normal appearance; class 2, non-specific changes; class 3 suspicion of malignant changes; and class 4, visible tumor. EBUS findings were classified as benign in cases where the normal

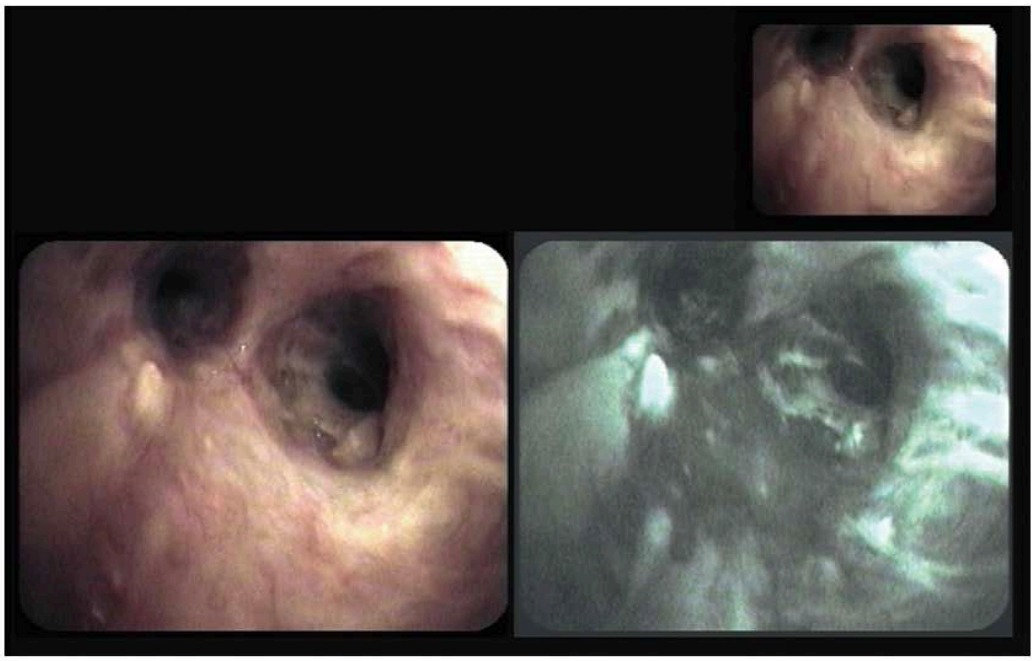

Figure 1 A case of central early-stage lung cancer (squamous-cell carcinoma) located in left upper lobe. Subtle changes of the mucosa on white light bronchoscopy are clearly depicted in the autofluorescence view (low autofluorescence).

seven-layer structure of the bronchial wall was preserved, or malignant in cases where thickening of the wall, destroyed layer structure, or parabronchial infiltration were found. Histologic examination of the lesions revealed that malignancy was correctly diagnosed by AF as class 3 in 69% and by EBUS in 97% of cases, while in benign lesions the correct diagnosis was obtained by AF in 55% and by EBUS in 92% of cases. The correlation coefficient for AF/histology was 0.59 and for AF + EBUS/histology 0.91. Thus, these authors proved that EBUS addition improves the positive predictive value of AFB alone in autofluorescence-positive lesions.

Endobronchial ultrasound and early central lung cancer staging

Surgery has been considered as a primary choice for the treatment of CELCs with an 80–90% 5-year survival rate.[18] As many CELC may develop in main or lobar bronchus, up to 30% of patients with CELC undergoing surgery will require bilobectomy or pneumonectomy, and the remaining 70% require lobectomy.[19] Therefore, surgery can imply a waste of normal lung parenchyma tissue in these patients, who usually have a long smoking history and present with a poor cardiopulmonary status. Moreover, synchronous lesions can be detected in up to 20% of patients and further metachronous lesions may develop in 14–30% of patients with CELCs.[17] In this setting preserving lung parenchyma is mandatory and other therapeutic options apart from surgery, such as bronchoscopic procedures, have to be considered.

Currently several therapeutic bronchoscopic procedures for CELC are available including: laser resection, argon-plasma coagulation, cryotherapy, brachytherapy, and photodynamic therapy (PDT). The decision to use endoscopy therapeutic intervention is based on the extent of the tumor invasion through the bronchial wall. Tumors with extracartilaginous invasion have been reported to have lymph node metastases in 6.4% of cases,[20] while lesions confined to the mucosa and submucosa are unlikely to have lymph node metastases.[21] Furthermore laser beams cannot penetrate the exterior wall of the cartilage. Following this approach it is crucial for the tumor to be confined within the mucosa and submucosa for successful endoscopic treatment.[22]

The conventional assessment of bronchial invasion has been based on bronchoscopical features and high resolution computer tomography (HRCT). Some authors have demonstrated a correlation between bronchoscopic tumor features and the depth of invasion. Konaka *et al.* analyzed surgical specimens in patients with CELC and reported that the greatest tumor dimension strongly correlated with the depth of intrabronchial invasion of the tumor.[23]

Over the last few years several studies have focused on the usefulness of EBUS in the assessment of tumor invasion (Fig. 2). In the first reported study by Kurimoto *et al.*,[7] EBUS

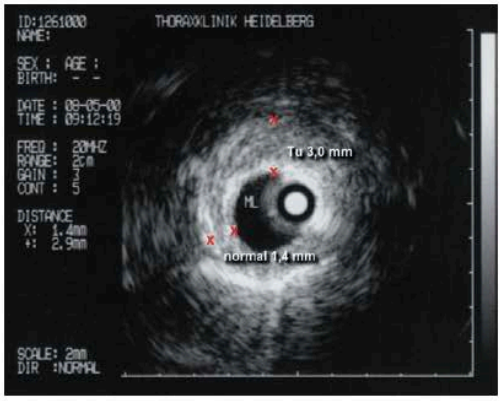

Figure 2 Echographical appearance of a tumor (Tu 3 mm) widening the mucosa (normal mucosa 1.4 mm). The tumor is not transgressing the bronchial wall.
Courtesy of Prof. Dr. Heinrich Becker (Heidelberg, Germany).

was performed "ex vivo" on 24 resected lung cancer specimens and EBUS images were then compared with histopathologic findings. The depth of the tumor invasion as determined by the ultrasonogram and the histopathologic findings was the same in 23 of 24 lesions (95.8%), but was overestimated on the ultrasonogram in the remaining lesion.

Tanaka *et al.*[4] were the first to compare EBUS findings performed "in vivo" with histopathological findings. They recruited 35 patients with intrathoracic malignancy cases where it had not been possible to clearly diagnose whether the tracheo-bronchial wall was involved with the tumor through routine chest diagnostic procedures, such as CT. Among 35 patients, 25 had tumors arising from the extratracheobronchial wall and the other 10 patients had intraluminal tumors. Fifteen patients underwent surgery and the diagnosis of EBUS was confirmed pathologically in 14.

In 2002 Miyazu *et al.*[22] performed EBUS on 12 patients that had 18 CELCs eligible for PDT. According to the evaluation by EBUS nine of these 18 lesions were diagnosed as intracartilaginous and were successfully treated with PDT. The remaining nine lesions were diagnosed as extracartilaginous based on the assessment by EBUS. Six of these nine patients were considered candidates for surgery. Resected tissue specimens revealed that the depth of the tumor invasion estimated by EBUS and the histopathologic findings were identical in six patients. Therefore they obtained confirmation of EBUS findings in all surgical specimens and positive follow-up for the others. These authors compared the EBUS findings with HRCT and demonstrated that EBUS provides more accurate information about the depth of tumor invasion than conventional WLB and HRCT.

Herth *et al.*[24] evaluated the contribution of EBUS in therapeutic bronchoscopy and found that in 28% of patients referred for presumed CELC, EBUS established disease extent, which could have made endoscopic curative treatment impossible.

In another series of 22 CELCs, Takahashi *et al.*[25] studied the ability of EBUS predicting the depth of cancer invasion. They determined the degree of the depth of tumor invasion by means of EBUS and classified the lesions into two groups: A: "the invasion does not reach cartilaginous layer," and B: "the invasion involves cartilaginous layer." Then the patients were treated with irradiation, PDT, or surgical resection and clinicopathological findings, and responses to the treatment were compared with the ultrasonographical classification. Of 14 group A lesions, 10 were treated with PDT, resulting in complete remission (CR) for nine lesions and no CR for one lesion. The other four lesions were surgically treated. The pathological examination revealed invasion beyond the cartilage in one lesion, while in the other three lesions invasion did not exceeded the cartilaginous layer. Of eight group B lesions, four underwent surgery and the pathological findings showed no cartilaginous invasion in one case and extracartilaginous invasion in the remaining three lesions. Two group B lesions were treated with PDT, resulting in CR for one lesion and no CR for the other. The remaining two group B lesions were treated with irradiation, resulting in CR for both. For these authors, in their evaluation of CELC depth invasion by means of EBUS, the sensitivity was 12/14 (85.7%), the specificity was 4/6 (66.7%), the accuracy was 16/20 (80%), and the positive predictive value was 12/14 (85.7%).

Although these authors demonstrated that EBUS is an accurate method for staging CELC and decisions of endoscopic treatments based on EBUS are very reliable, they nevertheless reported some problems with the technique:[7,22,25]

1 The visualization of lesions at bronchial spurs is difficult.
2 At 20 Hz the first marginal echo is more than 10 times thicker than the epithelium. Thus, in cases of CIS, they would not be visible on EBUS.
3 CELCs with large longitudinal extension: a radial probe only provides a single cross-section. In such cases, careful slow manipulation of the probe along the lesions is required for the observation of the whole tumor.
4 The need of a thick flexible bronchoscope (therapeutic bronchoscope) with a working channel over 2.8 mm.

In summary, a comprehensive approach using AFB and EBUS enables selection of the optimal therapeutic strategy for CELC. AFB has demonstrated usefulness in delineating the CELC margins and detecting multicentricities. Accurate evaluation of the depth of invasion is essential when deciding on the indications of endoscopic intervention. The evaluation of CELC depth tumor involvement by bronchoscopic features is subjective and requires skills. The evaluation of intrabronchial invasion by EBUS is feasible and superior compared with the prediction based on the interpretation of the endoscopic appearance. Advances in medical science such as new EBUS probes and optical coherence tomography[26] may contribute to better performance in the diagnosis and management of CELC.

Disclosure

No authors report any conflict of interest.

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Sanz-Santos et al. BMC Cancer 2012, 12:34
http://www.biomedcentral.com/1471-2407/12/34

BMC Cancer

RESEARCH ARTICLE Open Access

Contribution of cell blocks obtained through endobronchial ultrasound-guided transbronchial needle aspiration to the diagnosis of lung cancer

José Sanz-Santos[1,3*], Pere Serra[1], Felipe Andreo[1,4], Maria Llatjós[2,4], Eva Castellà[2,4] and Eduard Monsó[4,5]

Abstract

Background: Conventional smears of samples obtained by endobronchial ultrasound with real-time transbronchial needle aspiration (EBUS-TBNA) have proven useful in lung cancer staging, but the value of additional information from cell-block processing of EBUS-TBNA samples has only been marginally investigated. This study focussed on the contribution of cell block analysis to the diagnostic yield in lung cancer.

Methods: Patients referred for lung cancer diagnosis and/or staging by means of EBUS-TBNA were enrolled, the adequacy of the obtained samples for preparing cell blocks was assessed, and the additional pathologic or genetic information provided from cell block analysis was examined.

Results: In 270 lung cancer patients referred for EBUS-TBNA (mean age, 63.3 SD 10.4 years) 697 aspirations were performed. Cell blocks could be obtained from 334 aspirates (47.9%) and contained diagnostic material in 262 (37.6%) aspirates, providing information that was additional to conventional smears in 50 of the 189 samples with smears that were non-diagnostic, corresponding 21 of these blocks to malignant nodes, and allowing lung cancer subtyping of 4 samples. Overall, cell blocks improved the pathologic diagnosis attained with conventional smears in 54 of the 697 samples obtained with EBUS-TBNA (7.7%). Cell blocks obtained during EBUS-TBNA also made epithelial growth factor receptor mutation analysis possible in 39 of the 64 patients with TBNA samples showing metastatic adenocarcinoma (60.1%). Overall, cell blocks provided clinically significant information for 83 of the 270 patients participating in the study (30.7%).

Conclusions: Cell-block preparation from EBUS-TBNA samples is a simple way to provide additional information in lung cancer diagnosis. Analysis of cell blocks increases the diagnostic yield of the procedure by nearly seven per cent and allows for genetic analysis in a sixty per cent of the patients with metastatic adenocarcinoma.

Keywords: Cell block, Endobronchial ultrasound, Transbronchial needle aspiration, Lung cancer

Background

With the introduction of novel targeted therapies for non-small cell lung cancer (NSCLC), cytologists have had to cope with a corresponding rise in the need for accurate diagnosis and appropriate classification of subtypes. The analysis of genetic abnormalities in cancer cells, such as mutations in the epithelial grow factor receptor (EGFR) gene [1], has become crucial for the choice of treatment. Thus, conventional cytology staining does not always provide sufficient information and additional tissue is often required. The possibility of tailored treatments for lung cancer has come at the same time as the increased availability and use of minimally invasive sampling procedures, such as endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA). This technique can obtain both mediastinal and hilar cytological samples of nodes and masses that are appropriate for conventional smear and, in most cases, for immunohistochemistry [2].

Material recovered during EBUS-TBNA can be processed additionally as a cell block and made available for ancillary diagnostic procedures. The usefulness of

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cell blocks has been acknowledged in fine-needle procedures, and several medical societies have recently recommended its routinely use for lung cancer diagnosis [3,4]. This processing technique, however, is not yet widely used on EBUS-TBNA and there is little information about its contribution to the diagnostic process. The aim of this study was to evaluate that contribution in a prospectively recruited series of patients undergoing EBUS-TBNA for the diagnosis and/or staging of lung cancer.

Methods

Population

In North Barcelona Health Area all patients who had a suspicion of lung cancer are referred by the general practitioner to the Lung Cancer Unit for diagnosis. EBUS-TBNA was used as a diagnostic procedure in patients with mediastinal masses and/or nodes and with negative results from previous endoscopic procedures. EBUS-TBNA was additionally used for staging in all NSCLC patients who did not show distant metastasis at the first examination. The present study included all lung cancer patients who were diagnosed and/or staged by means of EBUS-TBNA between January 2006 and December 2009. A CT scan of the lung, mediastinum, and upper abdomen was performed in all cases using a multidetector-row spiral CT scanner (Marconi M8000, Phillips, Best, The Netherlands) in the month prior to staging, and nodes with a short-axis diameter greater than 10 mm in the scan were considered abnormally enlarged [5]. EBUS-TBNA was used for staging in all referred patients, independently of the size of the nodes in the scan, in accordance with previous reports that have showed the usefulness of EBUS-TBNA for the diagnosis of mediastinal metastasis in patients with a normal-appearing mediastinum at CT [6]. Patients with hemorrhagic diseases or coagulation disorders were excluded from staging by TBNA. The research protocol was approved by the regional ethics committee (Institut de Recerca en Ciències de la Salut Germans Trias i Pujol, reference: FIS PS09/01612) and all patients gave their signed consent to participation

EBUS-TBNA technique

EBUS was performed using a flexible bronchoscope (BF-UC160F-OL8, Olympus Optical Co Ltd., Tokyo, Japan) with a distal probe capable of producing linear parallel scans of the mediastinal and peribronchial tissues and a working channel suited to the performance of TBNA under direct ultrasound guidance. Local anaesthesia and conscious sedation were achieved using topical lidocaine spray and intravenous midazolam, respectively, in accordance with standard recommendations [7]. Mediastinal and lobar lung masses and nodes with a short-axis diameter of 5 mm or more [6] identified during the procedure were sampled under direct ultrasound visualization with a 22-gauge cytology needle specially designed for EBUS-TBNA (NA-201SX-4022, Olympus Optical Co Ltd.). The needle was guided beyond the bronchoscope channel to the tracheal lumen and then pushed forward from the sheath and inserted into the tracheal or bronchial wall under ultrasound guidance until the node or mass was reached. Once the needle tip was inside the target, negative pressure was maintained with a syringe at the proximal end of the catheter while the needle was pushed forth and back, releasing the suction before the needle was removed from the target structure.

Pathology

The aspirated material in the needle was recovered and the specimens were placed on slides and fixed with 95% ethanol. The slides were stained 1 minute with haematoxylin for rapid on-site evaluation by a cytopathologist; later the Papanicolau staining with orange A and eosin was completed at the pathology laboratory. An immediate assessment was given after each pass. The cytologist classified nodes as "normal tissue negative for malignancy" when the sample contained 40 lymphocytes per high-power field in cellular areas of the smear and/or clusters of pigmented macrophages, and no neoplastic cells [8], or as "metastatic" when recognizable groups of malignant cells were present. Nodes containing only isolated dysplastic cells were considered as "suspicious" but non-diagnostic. Nodes containing only bronchial or blood cells, which were considered as not representative of the structure that was the target of the sampling procedure, were also classified a non-diagnostic. In these situations the procedure was repeated up to 3 times and considered as useful for staging only when diagnostic samples were recovered from at least one of the aspirates [9,10]. The obtention of neoplastic cells from one lower paratracheal or subcarinal (stations 4R, 4 L and 7) node during sampling diagnosed N2 or N3 disease and precluded the performance of additional samplings in these regions. Stations showing only nodes with a short-axis diameter less than 5 mm during EBUS-TBNA were not sampled and labelled as normal, in agreement with previously published results [6].

Cell blocks were obtained and processed from the specimens recovered in the first pass whenever extra clotting material was available after the preparation of a minimum of four slides, or from a second or third passes when clotting material for cell blocks was not obtained in the previous passes, at request of the on-site cytopathologist. Cell blocks were obtained air-drying and clotting the specimens on filter paper and then placing them into 10% formalin just after for subsequent processing in the laboratory [11]. Cell blocks were

Sanz-Santos et al. BMC Cancer 2012, 12:34
http://www.biomedcentral.com/1471-2407/12/34

embedded in paraffin and sections of 5-μm thickness were obtained. Routine haematoxylin-eosin staining was used on cell-block sections and, when necessary, immunohistochemical stainings were applied for the identification or phenotyping of malignant cells. In cases of adenocarcinoma, somatic mutations of the genes coding the tyrosine kinase domain of EGFR were examined on cell-block samples, using methods previously described [12].

Statistical analysis

Data were introduced in a database and analyzed using SPSS software version 17.0 (SPSS Inc., Chicago, Illinois, USA). Results were expressed as absolute and relative frequencies for categorical variables, and as means and standard deviations (SD) or, when required, as medians and interquartile ranges (IQR), for continuous variables. First, availability of cell blocks containing adequate tissue samples from nodes or masses sampled by means of EBUS-TBNA was assessed. Second, the provision of new pathologic information from these cell blocks was analyzed. Information additional to pathology was defined as the establishment of a cytological diagnosis through the examination of the cell block from a sample with a previous non-diagnostic conventional smear or the determination of the NSCLC subtype based on the cell block when the smear diagnosis was NSCLC not otherwise specified (NSCLC-NOS). Finally, the impact of the additional information provided by the analysis of cell blocks over patient staging was assessed. The recovery of a cell block suitable for performance of genetic analysis of EGFR mutations in patients with metastatic adenocarcinoma was considered as additional genetic information. A p value of 0.05 or less was reported as statistically significant in the performed statistical tests.

Results

EBUS-TBNA was performed on 270 patients with a final diagnosis of lung cancer; the patient's mean age was 63.3 (SD 10.4) years and the male-to-female ratio was 6.7:1 (Table 1). EBUS-TBNA diagnosed metastasis in 130 out of 181 patients with evidence of enlargement in mediastinal nodes on the CT (71.8%), and in 14 of the 89 patients with a normal appearance of the mediastinum on the scan (15.7%).

Of 697 TBNA procedures performed, with an average of 2.6 TBNA per patient, 672 aspirations were from nodes and 25 were from mediastinal masses. The median short-axis diameter of the sampled nodes was 10 mm (IQR 7-15) and 562 (80.6%) of them were in the mediastinum. Two-hundred twenty-three smears (32%) led to a diagnosis of metastatic disease, 285 (40.9%) showed lymphocytes and were negative for malignancy, 15 gave isolated atypical cells (2.1%) and 174 (25%) gave only non-representative material (Table 2).

Table 1 Population characteristics (n = 270)

Age, mean (SD), years	63.3 (10.4)
Gender (men), n (%)	235 (87)
Mediastinal nodal enlargement at CT, n (%)	181 (67.0)
Pathologic diagnoses, n (%)	
Adenocarcinoma	106 (39.3)
Squamous-cell carcinoma	65 (24.1)
Large cell carcinoma	10 (3.7)
NSCLC not otherwise specified	59 (21.8)
Small cell lung cancer	29 (10.7)
Atypical carcinoid	1 (0.4)

CT: computed tomography
NSCLC: non-small cell lung cancer

Cell blocks could be prepared from 334 aspirates (47.9%) obtained from 321 nodes and 13 mediastinal masses and adequate material for diagnosis was recovered from 262 (37.6%) of them (Figure 1). The median short-axis diameter of nodes from which material for cell block processing was obtained was 11 mm (IQR 8-15), a size which was larger than the size of nodes that did not give material suitable for cell blocks after three passes (short-axis diameter 9 [IQR 7-14]) ($p < 0.001$, Mann-Whitney U test). Most of the samples with a cell block available were obtained from nodes located in the mediastinum, mainly in the subcarinal region (49.1%). Malignancy was diagnosed at the examination of 122 of the obtained cell blocks, being the block sample diagnostic and negative for lung cancer in 130 of the performed aspirations. In 10 cases the cell block showed only isolated atypical cells and was considered non-diagnostic.

Cell blocks provided additional pathologic information in 50 cases out of the 189 (26.4%) smears from samples that were non-diagnostic or that showed only isolated atypical cells (Figure 1). Twenty-one of these blocks corresponded to malignant nodes and 29 to normal nodes. Thus, information from cell blocks raised the overall

Table 2 Diagnoses in conventional smears of transbronchial needle aspirates (n = 697)

Squamous cell carcinoma	29 (4.2)
Adenocarcinoma	98 (14.1)
NSCLC not otherwise specified	63 (9.0)
Small cell carcinoma	33 (4.7)
Normal tissue	285 (40.9)
Non-diagnostic	
Isolated atypical cells	15 (2.1)
Non-representative	174 (25.0)

NSCLC: non-small cell lung cancer

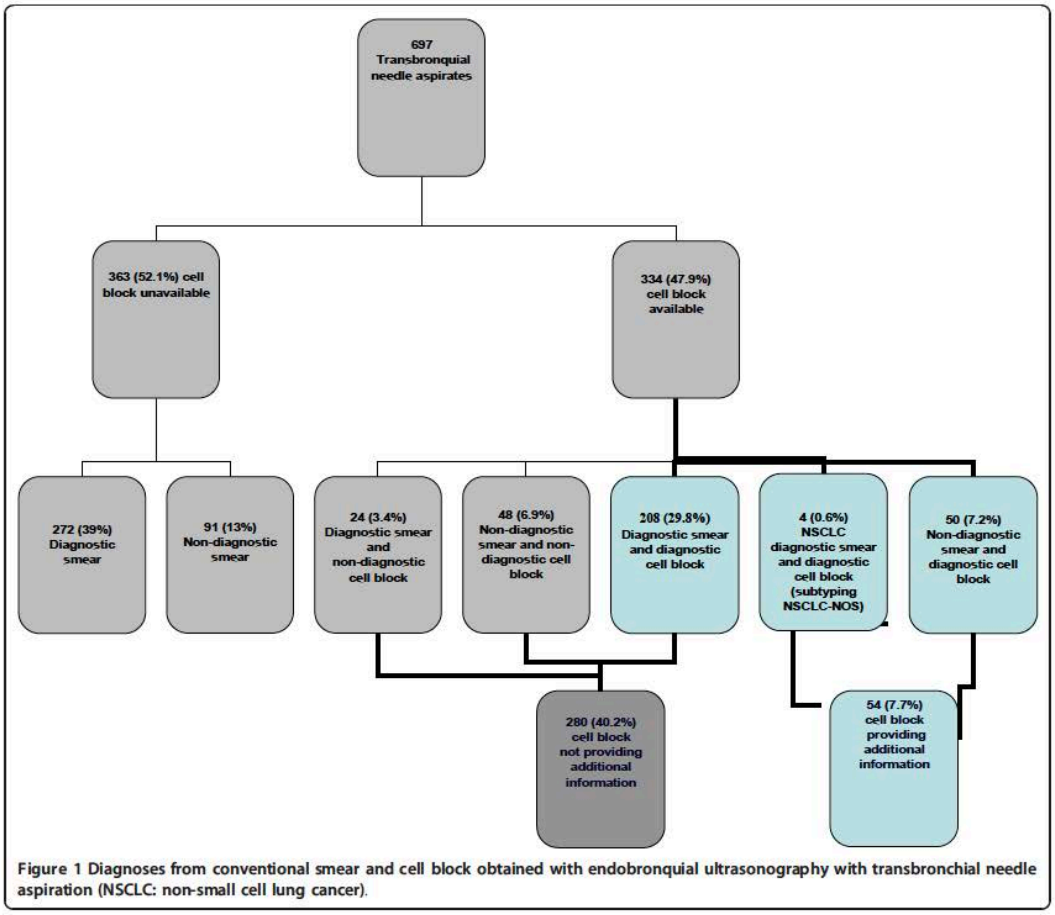

Figure 1 Diagnoses from conventional smear and cell block obtained with endobronquial ultrasonography with transbronchial needle aspiration (NSCLC: non-small cell lung cancer).

diagnostic yield of EBUS-TBNA through an increase in the number of the diagnostic samples from 508 (72.9%) to 558 (80%). There were 63 cases of NSCLC-NOS on the conventional smear, in 4 (6.3%) of those the cell block achieved the subtype providing also additional pathologic information

Cell blocks obtained during EBUS-TBNA provided clinically significant information for 83 of the 270 patients participating in the study (30.7%). Pathologic diagnosis was attained in some nodes only through cell block processing in 40 patients (14.8%), and cell block was the only sample that demonstrated mediastinal metastases in 7 of them. In 4 patients with conventional smears showing NSCLC-NOS, cell blocks allowed the identification of the sub-type of the NSCLC. Additionally, cell blocks provided material suitable for EGFR gene mutation analysis in 39 of the 64 patients with metastatic adenocarcinoma in the sampled nodes (60.1%), and allowed the identification of a mutation of the EGFR gene in two patients.

Discussion

Cell blocks prepared from EBUS-TBNA material in our series contained diagnostic material in a third of the samplings and provided additional information to non-diagnostic smears, increasing the accuracy of EBUS-TBNA by a seven percent, to a diagnostic yield of 80%. Cell blocks obtained during EBUS-TBNA provided clinically significant information for one third of the patients participating in the study (30.7%), through accurate typing of the disease, identification of metastasis in the mediastinum, and, in patients with adenocarcinoma, EGFR genetic analysis in cell block samples.

Sanz-Santos et al. BMC Cancer 2012, 12:34
http://www.biomedcentral.com/1471-2407/12/34

With the development of novel treatments for NSCLC that have different degrees of efficacy and toxicity in NSCLC subtypes, an accurate pathologic classification has become essential. Most patients with NSCLC present with advanced non-operable disease and surgical biopsies allowing additional pathologic and genetic analyses are not available [13]. The difficulties of pathologic diagnosis have increased with the emergence of minimally invasive procedures like EBUS-TBNA. This technique provides conventional smears for cytology that have a good correlation with histological diagnoses. Feller-Kopman and colleagues [14] compared the cytological samples obtained by EBUS-TBNA with core biopsies or surgical excision samples in a series of 88 patients, finding that diagnoses were equivalent in most patients. Cell blocks can be obtained by means of EBUS-TBNA, and, compared with conventional smears, allow the performance of sections suitable for larger immunohistochemical staining batteries [15,16]. When cell blocks prepared with EBUS-TBNA material are used for NSCLC subtyping, the adequacy of tumour tissue available for immunohistochemistry is a key issue [17]. That topic can be easily managed when the recovered samples are subject to rapid on-site evaluation, as in our study; thus the immediate evaluation of the sample increases the diagnostic yield and decreases the need for unnecessary repeated diagnostic procedures [18]. The on-site cytopathologist confirms the adequacy of the recovered material, minimizing the rate of unsatisfactory samples and requests for further sampling when additional material is needed for cell blocks. Following this approach 4 (6.3%) cases initially diagnosed as NSCLC-NOS on the conventional smear could be adequately subtyped in our study.

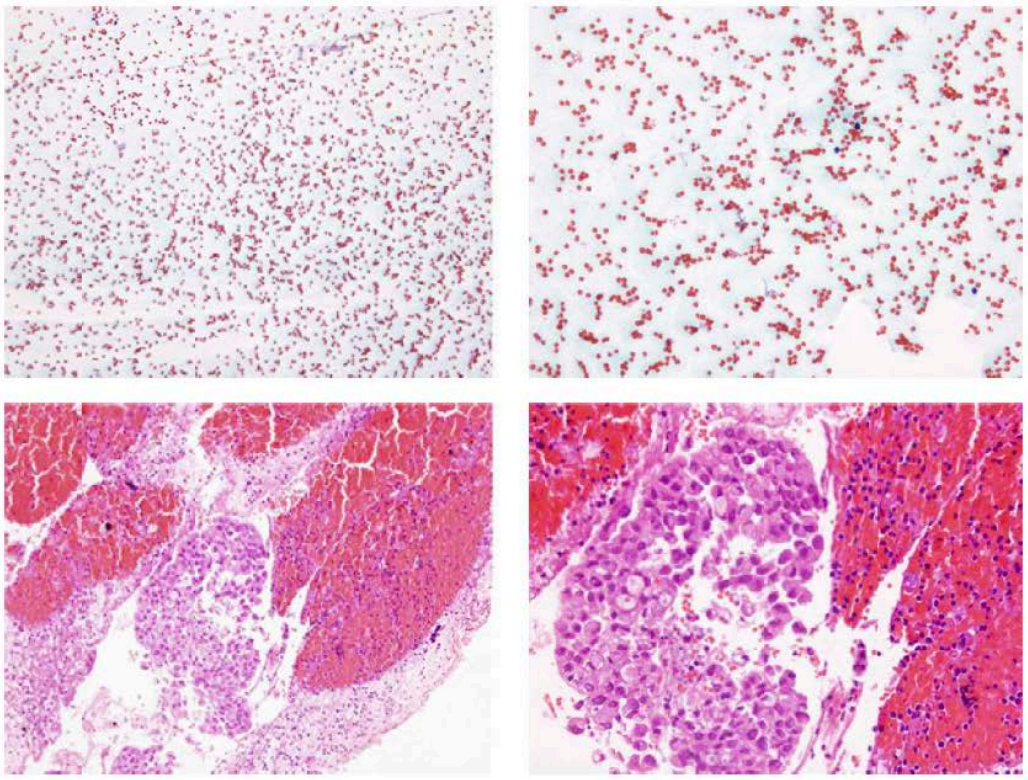

Figure 2 Fine needle aspiration from a subcarinal node in a case of metastatic adenocarcinoma. Non-diagnostic conventional smear (figure 2a and b) and cluster of adenocarcinoma cells in the the cell block (figure 2c and d). **a**: Unsatisfactory specimen. Blood cells in an otherwise acellular smear (Papanicolau stain, x 100). **b**: Unsatisfactory specimen. Blood cells in an otherwise acellular smear (Papanicolau stain, x 200). **c**: Cluster of adenocarcinoma cells in a cell block (Hematoxilin and eosin stain, x 100). **d**: Cluster of adenocarcinoma cells in a cell block (Hematoxilin and eosin stain, x 200).

We found that over a 75% of the recovered cell blocks contained diagnostic cellular material, a percentage similar to those in other series where cell blocks from needle core biopsies have been processed [3,19], but lower than the figure attained by conventional smears [18]. Cell-block analysis achieved the diagnosis in 50 cases out of the 189 samples (26.4%) in which conventional smears were non-diagnostic in our study. Thus, with cell-block processing, the diagnostic yield of EBUS-TBNA rose from 72.9% to 80%. Twenty-one of these diagnostic cell blocks were from malignant nodes that would not have been diagnosed if the blocks had not been obtained and clinically implies that 7 patients were diagnosed of mediastinal metastases (N2/N3 disease) solely by the cell block analysis. We attribute this increase in the diagnostic yield mainly to the contribution of cell blocks to haematic non-diagnostic smears (Figure 2). One of the obstacles that bronchoscopists and cytopathologists have to deal during an EBUS procedure is a vascularised node; these nodes are more likely to contaminate the samples with red blood cells. In this situation the on-site cytopathologist may not be able for a proper diagnosis of the slides. These aspirates, processed as cell blocks, can be examined later on the pathology laboratory and sometimes harbour clusters of lymphocytes or malignant cells. Other situation apart from blood contamination is nodes or masses containing necrotic material.

Cell-block processing allowed for the performance of EGFR mutational analysis in 60% of our patients with a diagnosis of metastatic adenocarcinoma and in two of them confirmed the presence of an EGFR mutation, which confer sensitivity to the tyrosine kinase inhibitors gefitinib and erlotinib [20]. These findings agree with the few smaller studies that have focussed on the ability of EBUS-TBNA to obtain samples for EGFR gene mutation screening [21,22]. Nakajima and cols. [21] used this approach in a series of 46 patients with adenocarcinoma, detecting 11 patients with EGFR mutations. García-Olivé and cols. [22] found nodal metastasis by means of EBUS-TBNA in 36 patients from a series of 51 patients with this diagnosis; these authors recovered cell blocks that were adequate for EGFR analysis through EBUS-TBNA for most of their patients, and were able to identify mutations in two of them. Other cancer-related genetic mutations may also be predictive biomarkers, and their detection in TBNA samples might be useful for choosing a lung cancer therapy [1]. In this new scenario our study confirms the value of cell-block processing of the material recovered from malignant nodes using EBUS-TBNA.

In summary, cell-block preparation is a simple method that provides important additional information after EBUS-TBNA in lung cancer. In our study, it was possible to preserve diagnostic material for cell blocks from more than a third of the performed aspirates. This material supplemented the information from conventional smears in a third of the cases and increased the diagnostic yield of the technique by a seven percent. Overall, cell-block processing provided clinically significant information for on third of the lung cancer patients, and allowed for the performance of genetic analyses of EGFR mutations in a half of the samples showing metastatic adenocarcinoma, confirming the advantages of this processing method for the diagnosis and staging of lung cancer.

Acknowledgements
This study has been funded by grant from the Spanish government (CibeRes and FIS PS09/01612) and the Catalan Pneumology Foundation (Fundació Catalana de Pneumologia, FUCAP) and the Catalan Pneumology Society (SOCAP).

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Authors' contributions
JSS performed EBUS-TBNA, analyzed the data and wrote the original. PS performed EBUS-TBNA and acquired the data FA performed EBUS-TBNA and revised the final text. EC carried out the cytological examination. MLL carried out the cytological examination. EM performed EBUS-TBNA, designed the study and revised the final text.

Competing interests
The authors declare that they have no competing interests.

Received: 24 March 2011 Accepted: 21 January 2012
Published: 21 January 2012

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Pre-publication history
The pre-publication history for this paper can be accessed here:
http://www.biomedcentral.com/1471-2407/12/34/prepub

doi:10.1186/1471-2407-12-34
Cite this article as: Sanz-Santos *et al.*: **Contribution of cell blocks obtained through endobronchial ultrasound-guided transbronchial needle aspiration to the diagnosis of lung cancer.** *BMC Cancer* 2012 **12**:34.