BAL 검체 수집 연구

2017-08-19 하계 분자폐암연구회 임상연구 워크숍

건국대학교 병원 호흡기내과 김희정

Contents

Bronchoalveolar lavage (BAL)

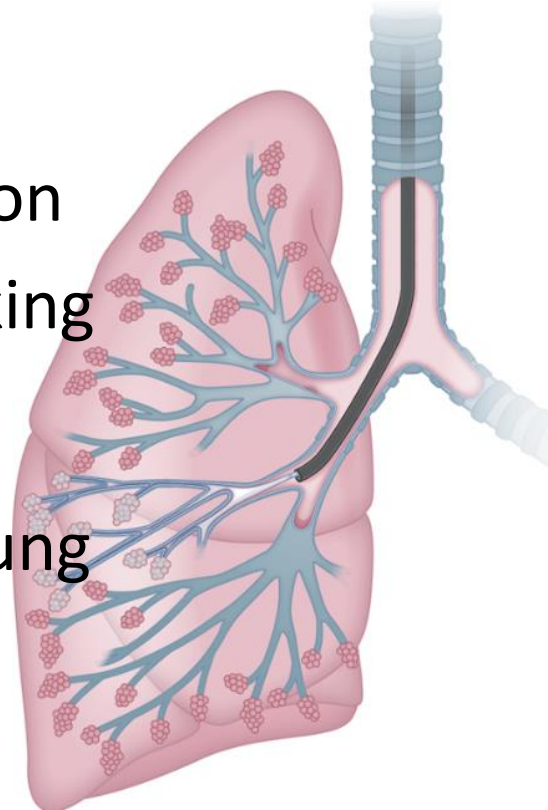
- In 1974, BAL was introduced as a research tool

J Lab Clin Med. 1974 Oct;84(4):559-73.

Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage.

Reynolds HY, Newball HH.

- Performed during flexible bronchoscopy
- **Minimally invasive method** that provides important information about immunologic, inflammatory, and infectious processes taking place at the alveolar level
- Obtain specimens from anatomically distinct locations of the lung



BAL fluid

- Allows the recovery of both cellular and non-cellular components from the epithelial surface of the **lower respiratory tract**
  - Representative of **the inflammatory and immune system** of the entire lower respiratory tract

  ↔ **Bronchial washings**; aspiration of either secretions or small amounts of instilled saline from the **large airways**

- BAL is a widely used technique applied to every area of pulmonary medicine

Clinical utility of BAL fluid

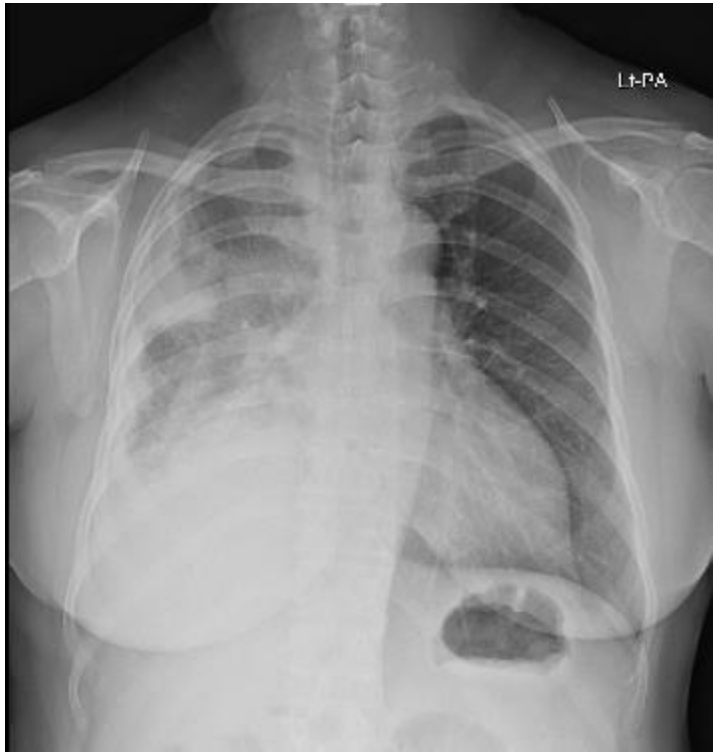
Test		Differential diagnosis
Clinical practice	Gross appearance	alveolar hemorrhage, alveolar proteinosis
	Microbiology	pneumonia
	Cytology	lung cancer
	Cell count and differential count	eosinophilic lung disease, interstitial lung disease
	Immunological parameters	sarcoidosis, hypersensitivity pneumonitis
	Polymerase chain reaction	*pneumocystis* pneumonia, tuberculosis
	Electron microscopy	pulmonary Langerhans cell histiocytosis
Researches	Various biochemical mediators related to pathological processes	
	Tissue markers	
	Flow cytometry	
	DNA probes	

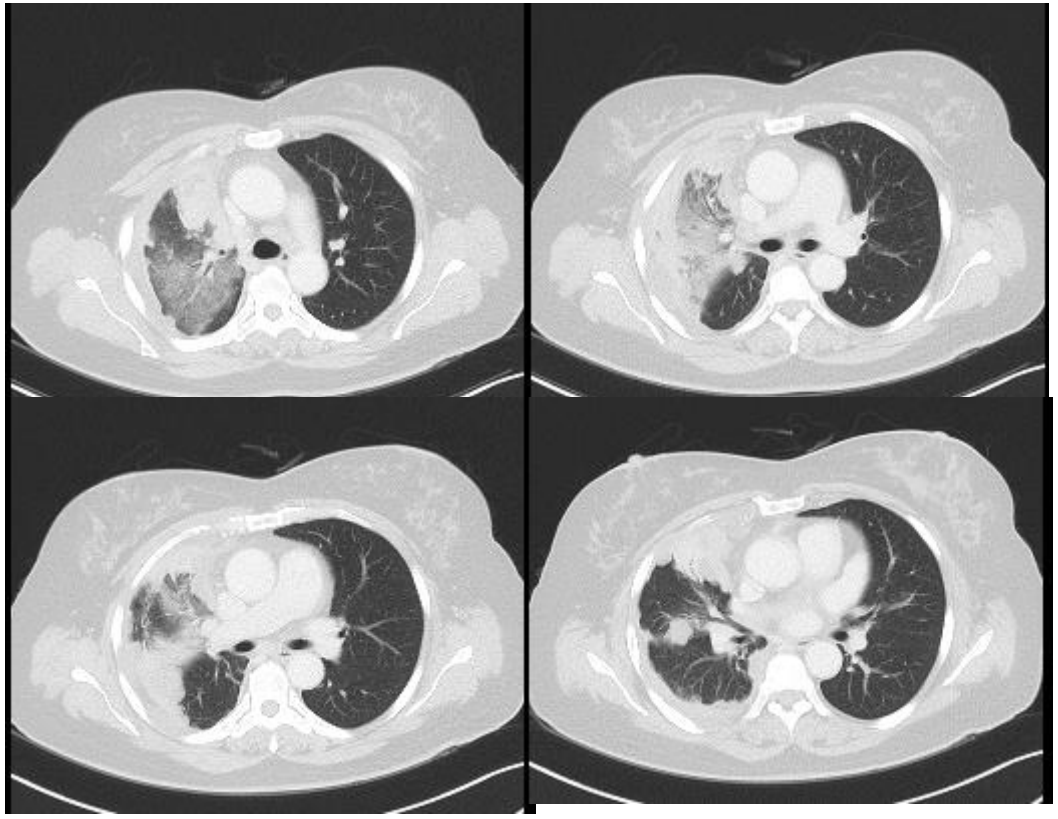
Advantages

- **Safety**
  - Even in critically ill patients, when biopsy or brushings may be contraindicated because of the risk of bleeding
- The yield of cells and fluid for analysis is **rarely inadequate** and often far exceeds cell numbers that can be obtained with small biopsies
- The cellular constituents in BALF are thought to **reflect parenchymal changes** on lung biopsy
- BAL is often performed for clinical purposes, permitting use of routinely discarded samples for research.

Case

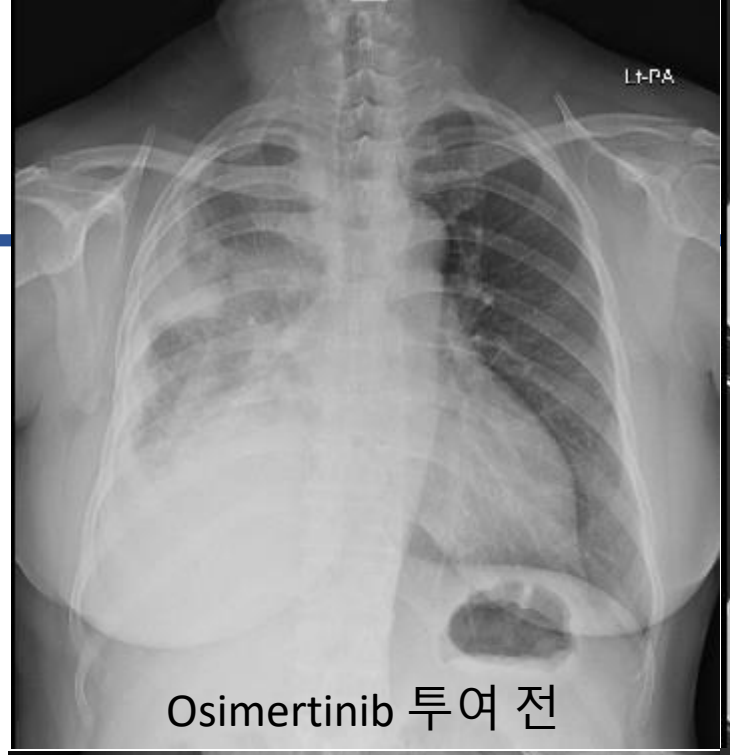
- ▶F/59
- ▶NSCLC, adeno ca., T4N2M1a stage IV, 19 del +
- ▶2013-07-22~2016-04-22 : progression after 1st line gefitinib

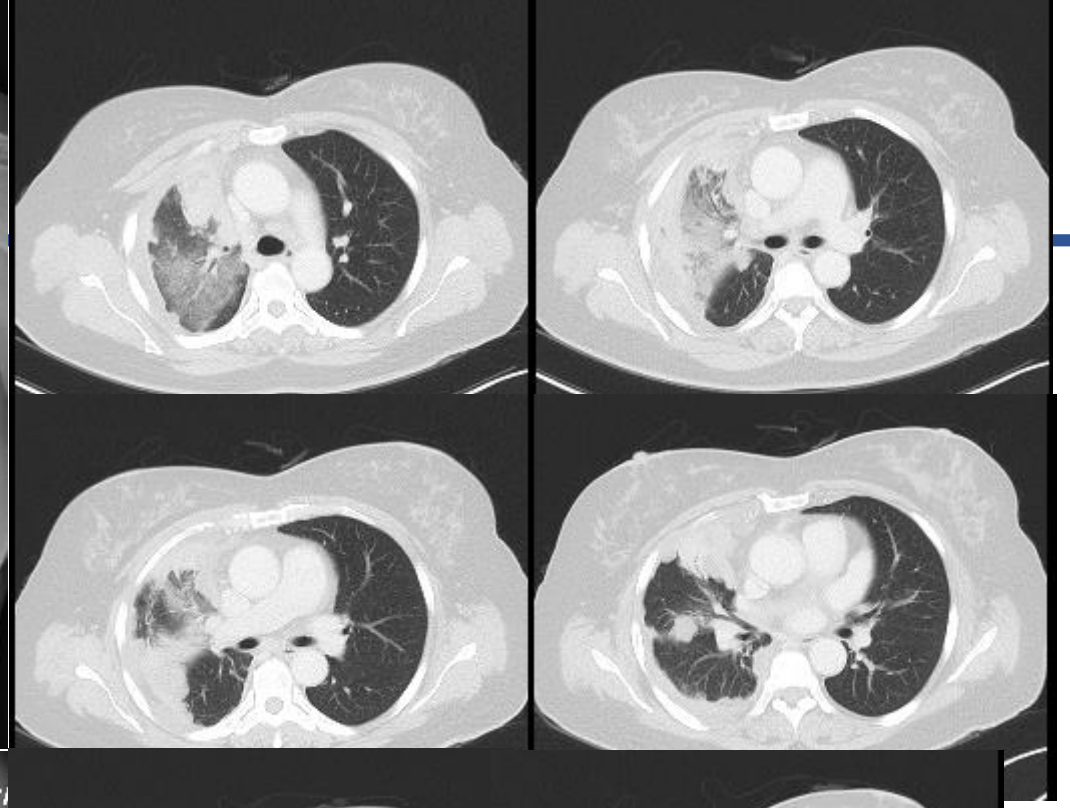


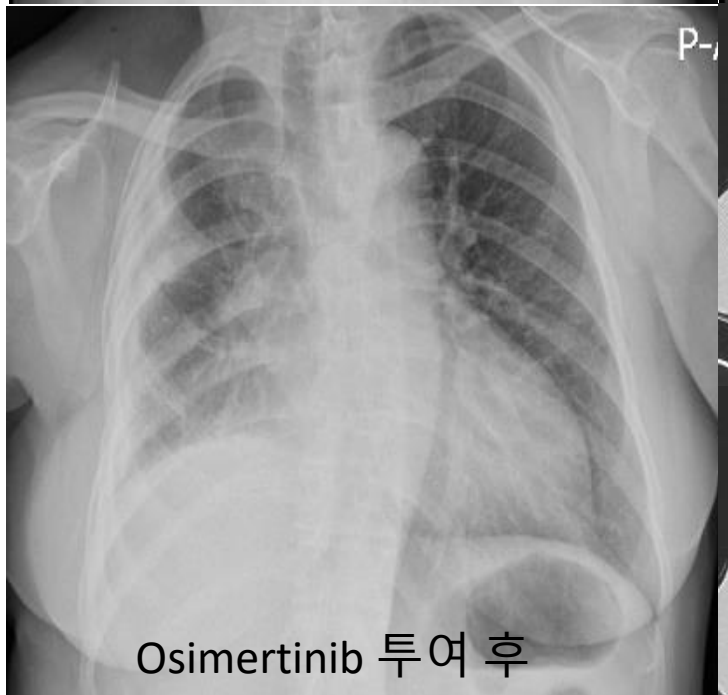


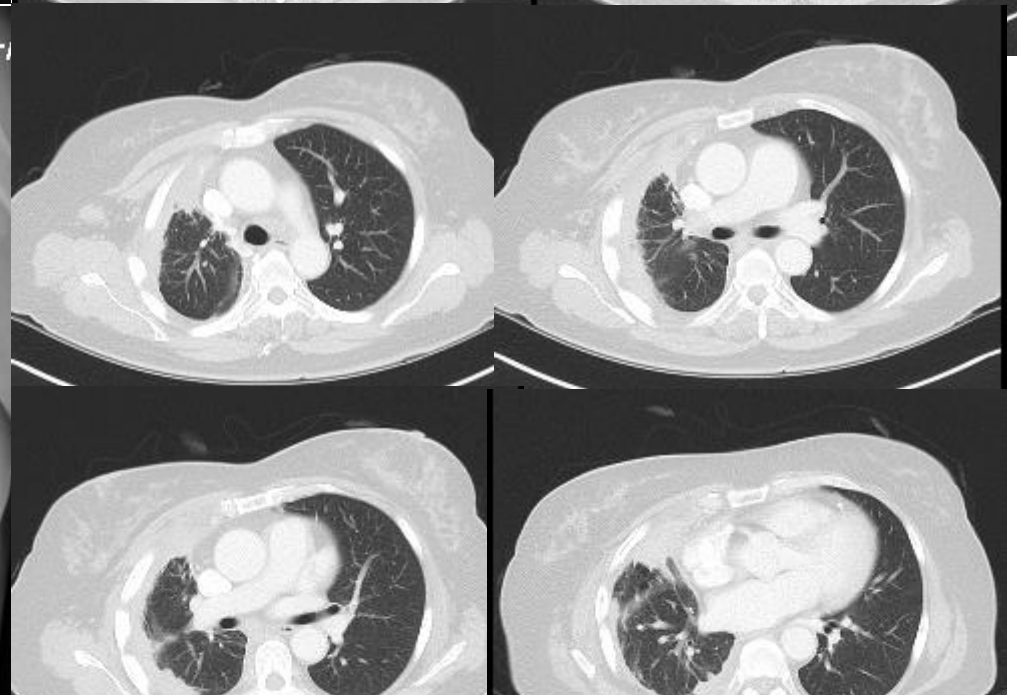
Case

- PCNBx. : Non-neoplastic lung parenchyme including vessel
- TBLB : Non-neoplastic lung parenchyme
- **BAL fluid cytology : NSCLC, favor ADENOCARCINOMA, 19 del, T790M**
- VATS Bx.
  - ADENOCARCINOMA, acinar (40%) and micropapillary (60%)
  - Exon 19 : Deletion : c. 2236_ 2249del., p. E746 _A750del
  - Exon 20 : Mutation : c.2369 C>T; p.Thr790Met **(T790M)**
- Enrolled into a clinical study (osimertinib)


Osimertinib 투여 전




Osimertinib 투여 후



Limitations (1)

- **Not completely risk free** and not all eligible subjects are willing to undergo an invasive procedure, which introduces bias
- Vast differences between subjects with **different environmental exposures**, particularly tobacco smoke
- Lack of **technique standardization** ; a few consensus reports
  - Uncertainties of the BAL process
  - European Respiratory Society (ERS) :1989, 1999
  - American Thoracic Society (ATS) ; 2007, 2012 (ILD)

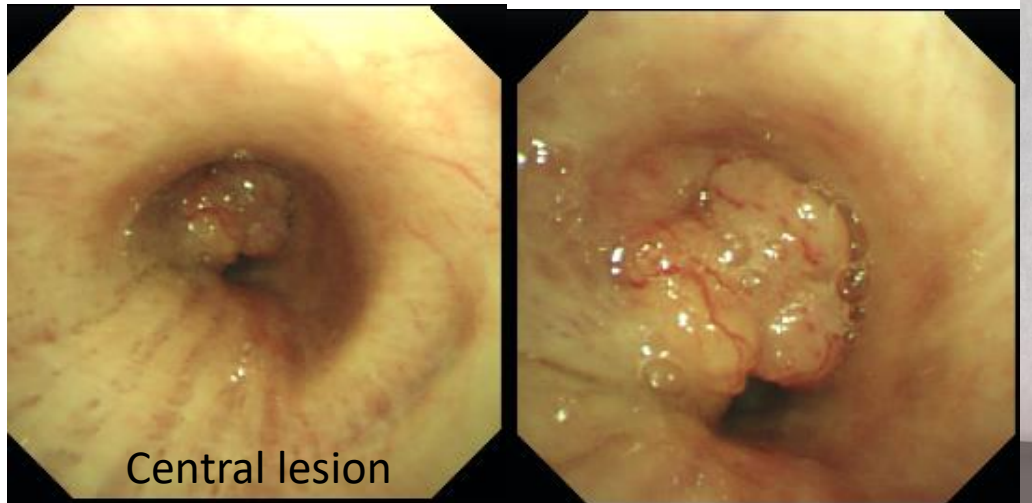
Limitations (2)

- Lack of a definitive **dilution marker**
  - The dynamic interaction of the alveolar and vascular space especially impacts **non-cellular components** such as cytokines

  → Proposed simultaneous BAL fluid and serum measurements for interpretation

- Contamination of the recovered fluid with **excess blood**
  - After routine inspection/ examination of the tracheobronchial tree

  → Before biopsy or brushings



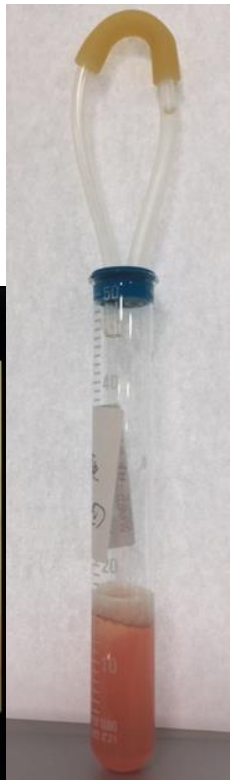


Table 1 Aspects Affecting Bronchoalveolar Lavage Results

Source of Variability	US BAL Cooperative[8]	European Respiratory Society 1999[6]	American Thoracic Society 2007
Disease process itself	Stated	State underlying disease	State underlying disease
Suction pressure during the procedure	"Gently" aspirate by handheld syringe	Keep to a minimum (25–100 mm Hg)	Keep below 100 mm Hg; avoid visible airway collapse
The handling of fluid: filtered/ nonfiltered; concentrated	No comment	State technique specifically	No filtering with gauze
Volume instilled	240 mL	Instill at least 100 mL	Instill at least 100 mL
Handling of first aliquot recovered	Pooled all samples	Specify	Pool all samples unless specified
Number of aliquots	Four	Specify and standardize	Specify and standardize
Position of patient	Semirecumbent	Specify	Specify
Area that is lavaged	Right middle lobe/lingula	Specify	Specify
Number of areas lavaged	One	Specify	Specify
Variability of lavage return	Discontinued lavage if difference between instilled and aspirated was > 100 mL	Report volume and percent of fluid returned; establish minimal percent recovered	Report volume and percent of fluid returned; at least 5% of instilled volume must be recovered
Reporting measurements of acellular components	Report per mL of fluid recovered	Report per mL of fluid recovered	Report per mL of fluid recovered
Sample storage	Specified	Specify	Specify

Baughman RP. Semin Respir Crit Care Med. 2007;28(5):475-85.

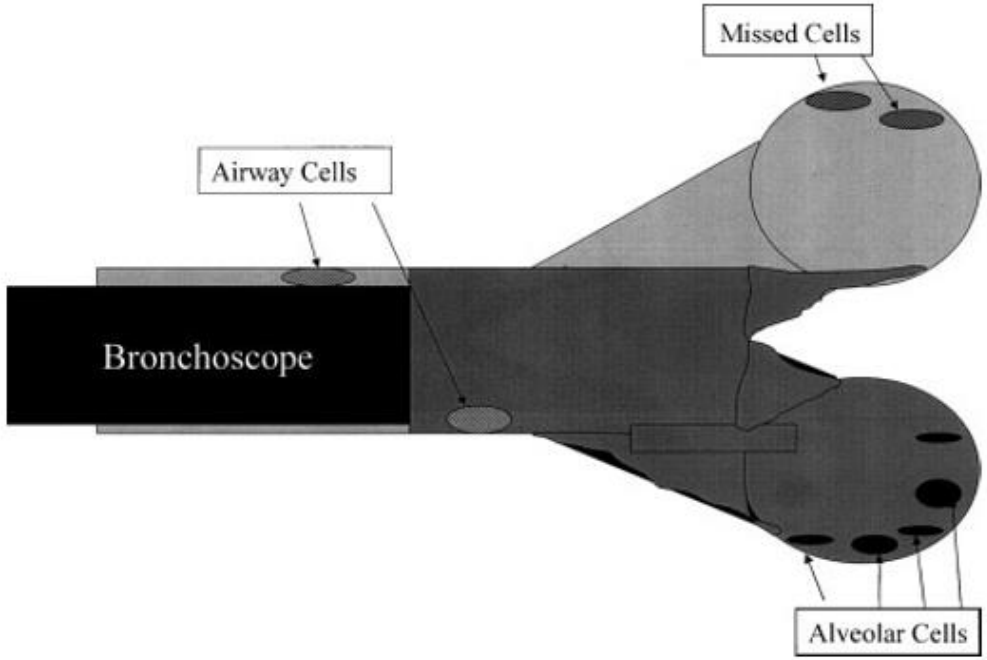
Technical aspects (1)

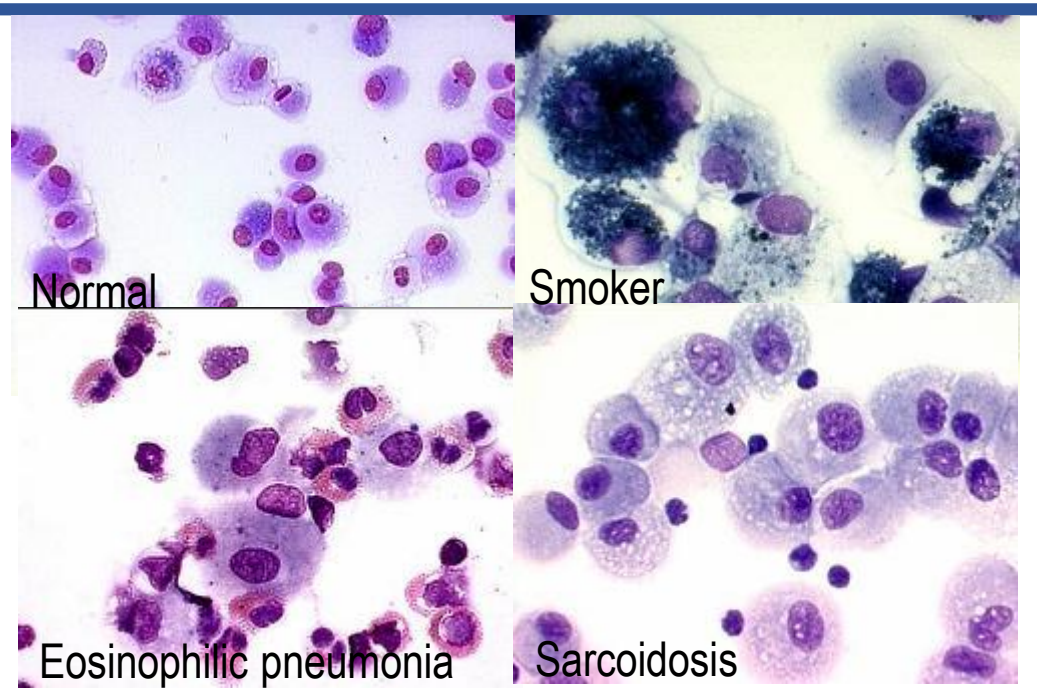
- Using a clean suction channel
- Tip of the bronchoscope is advanced distally until it is wedged (advanced to point of resistance and/or collapse of airway with gentle suction) into a subsegmental bronchus
  - The wedge position is usually at the level of the fourth to fifth branching
- Care should be taken to avoid trauma and coughing because these may lead to excessive contamination of the recovered fluid with mucus and blood
- In diffuse disease, segments of the lingula or the RML are routinely lavaged
- When localized disease is present radiographically, lavage should be carried out at the area of radiographic abnormalities, because the BAL results may be most abnormal from these areas

Technical aspects (2)

- After the bronchoscope is wedged, sterile saline is infused with a syringe into the suction port of the bronchoscope
- Mixed data are found regarding the use of warmed saline
  - Prewarming the lavage fluid to 37˚C may help prevent coughing and bronchospasm (esp. in patients with hyper-responsive airways) and may increase fluid recovery and cellular yield in comparison to instillations of fluid at RT
- Most institutions use aliquots of 20 to 60 mL
  - No significant data to support a specific aliquot size
  - The fluid is then removed from the lung by the use of negative pressure from a suction apparatus and collected into a specimen trap
- The suction channel of the bronchoscope should be maintained in the center of the airway lumen

Populations of cells in BAL fluid

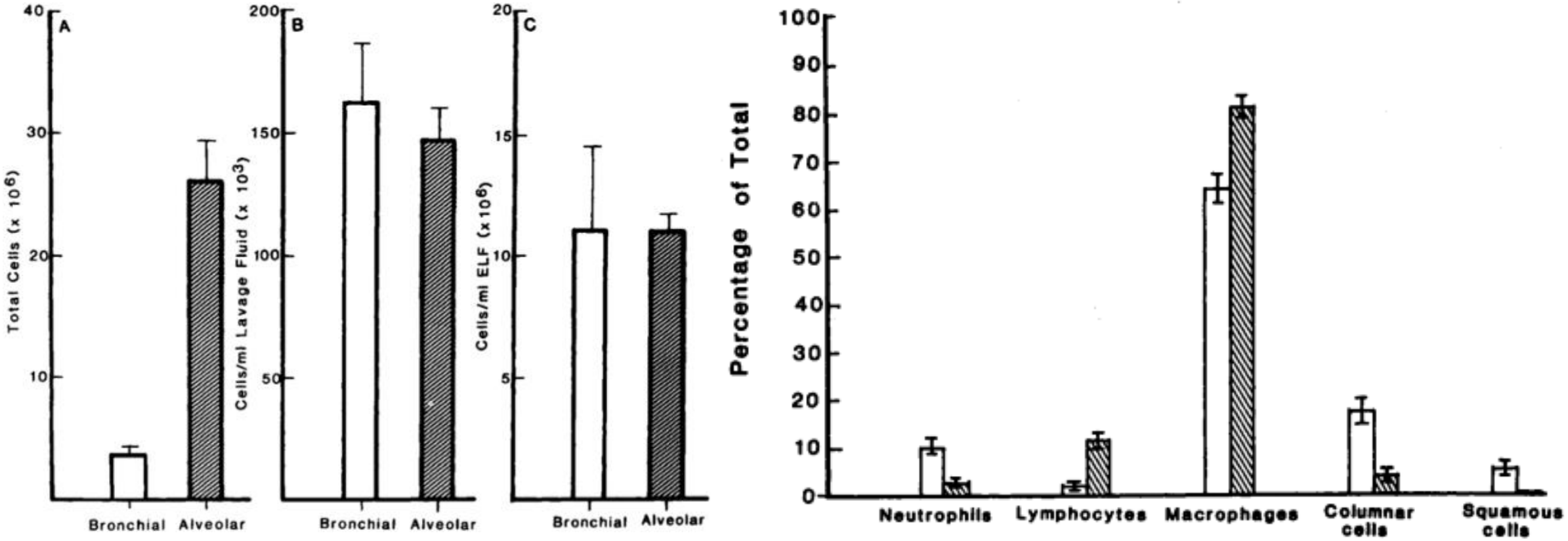




1) Cells in the alveoli that are within the BAL fluid → sampled
2) Alveolar cells that are not in contact with the BAL fluid → not sampled
3) Airway cells that may also be washed into the bronchoscope during the lavage process → sampled

Baughman RP. Semin Respir Crit Care Med. 2007;28(5):475-85.

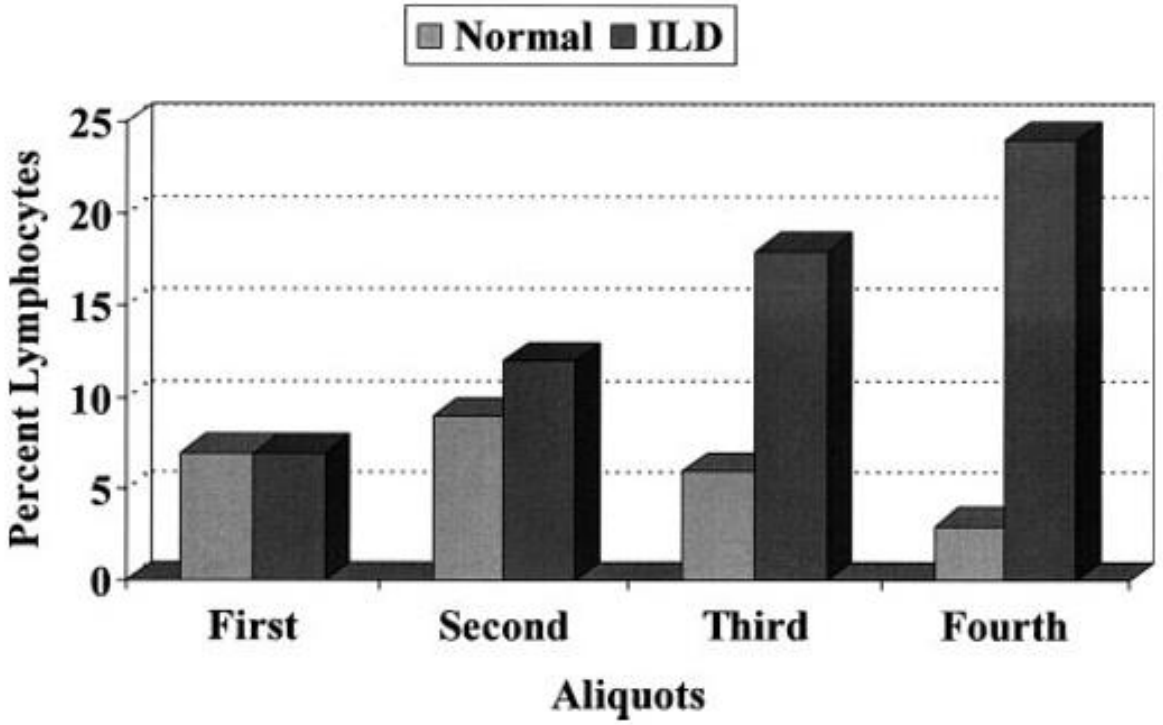
Populations of cells in BAL fluid



- Initial BAL aliquot (first 20 mL) primarily represents a ‘‘different compartment’’
- More specifically, a bronchial airway compartment
- Higher proportion of ciliated epithelial cells, more neutrophils, fewer lymphocytes, and more immunoglobulin A (IgA) when compared with subsequent instillations

Rennard SI et al. Am Rev Respir Dis. 1990;141(1):208-17.

Percentage of lymphocytes in the aspirated fluid



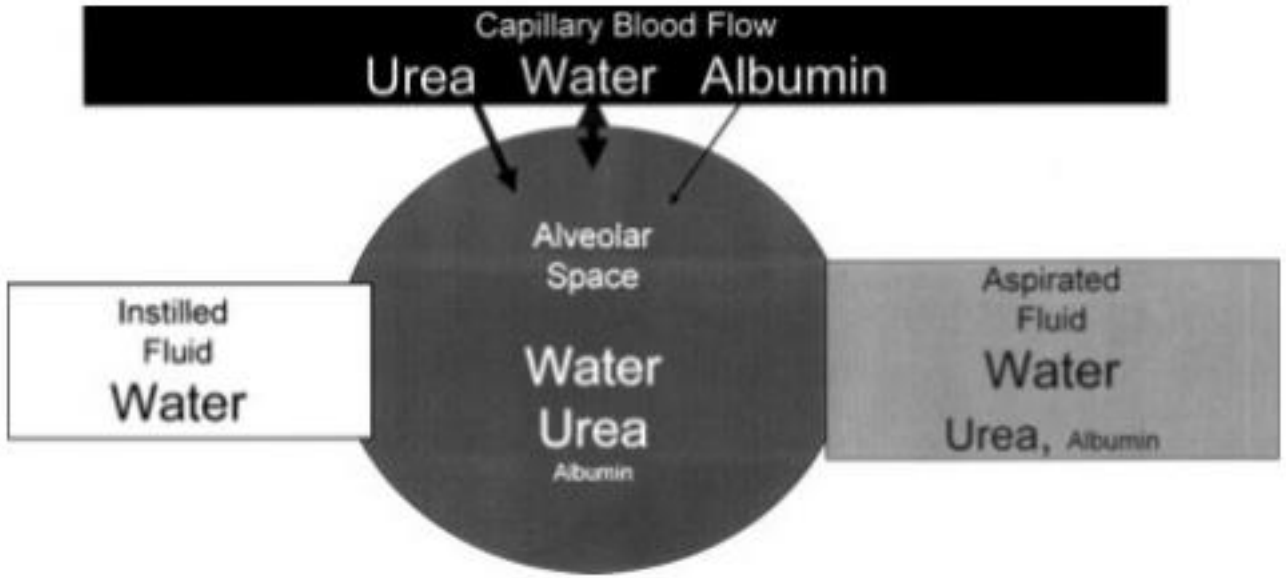
Healthy volunteers vs. **patients with ILD**, mostly sarcoidosis

►Percentage of lymphocytes in BAL fluid after each 60 mL aliquot of a BAL

►The percentage of lymphocytes is significantly higher after the second aliquot (a total of 120 mL instilled)

Baughman RP. Semin Respir Crit Care Med. 2007;28(5):475-85.

Various compartments involved in BAL fluid

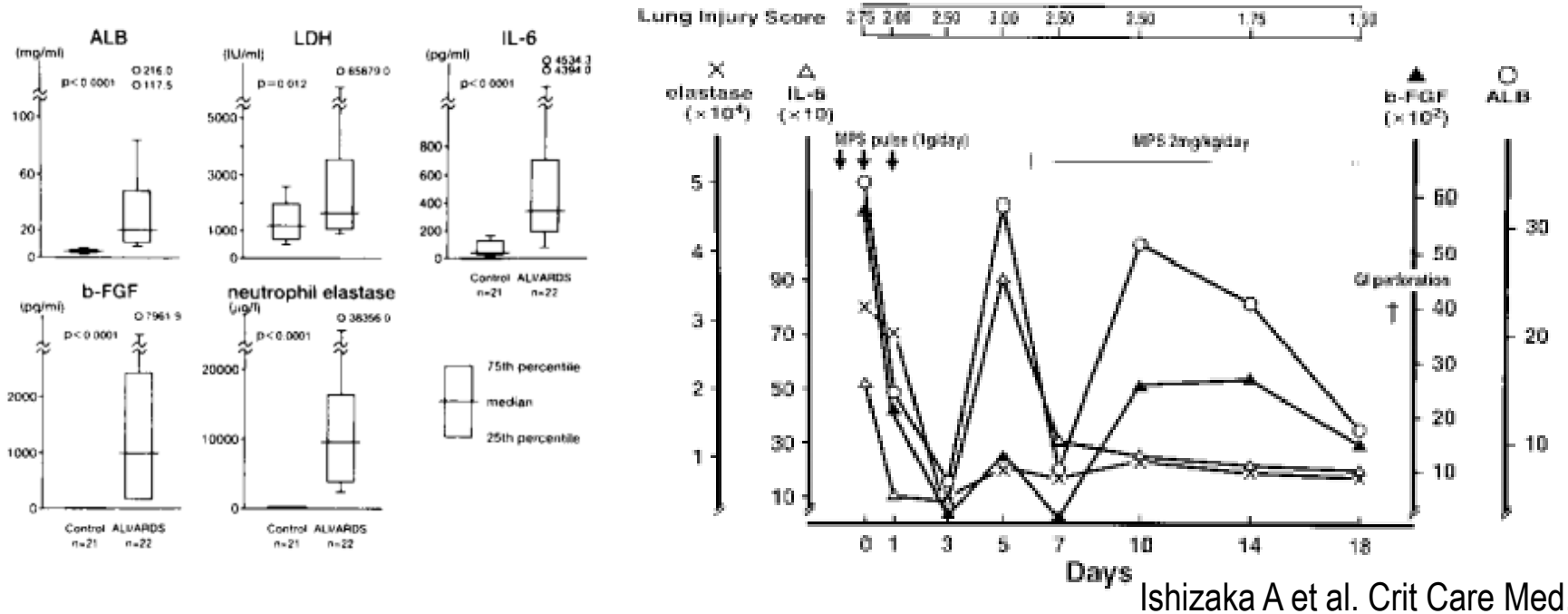


- ►Concentration of sodium chloride is the same in all four areas → no change
- ►Concentration of **urea** : capillary blood ≒ alveolar space
  - Urea in the capillary blood passes easily into the alveolar space
- ►Concentration of **albumin** : blood >> alveolar space
  - Albumin crosses more slowly into the space

Baughman RP. Semin Respir Crit Care Med. 2007;28(5):475-85.

The uncertainly of dilution

- New bronchoscopic microsample (BMS) probe
  - Consists of a **small cotton probe** attached to a **stainless steel** guidewire protected by a plastic sheath
  - To directly measure epithelial lining fluid



Ishizaka A et al. Crit Care Med. 2001;29(4):896-8.

- Soluble BAL constituents be reported **per mL of BALF** or as a **fraction of total component** present

Research trends using BALF in lung cancer

- Early researches; Diagnostic value of BALF for diagnosis of lung cancer

1808. [Combination of transbronchial **lung** biopsy and **bronchoalveolar lavage** in disseminated **lung** diseases].
Dürschmied H, Wiesner B, Liebetrau G, Haenselt V, Jäger J, Lenich R.
Z Erkr Atmungsorgane. 1985;165(3):220-7. German.
PMID: 4090536

1810. **Bronchoalveolar lavage** in lymphangitic spread of adenocarcinoma to the **lung**.
Fedullo AJ, Ettensohn DB.
Chest. 1985 Jan;87(1):129-31.
PMID: 3965259

1812. **Bronchoalveolar lavage** diagnosis of bronchiolo-alveolar carcinoma.
Sestini P, Rottoli L, Gotti G, Miracco C, Luzi P.
Eur J Respir Dis. 1985 Jan;66(1):55-8.
PMID: 2984034

460. **Bronchoalveolar lavage** and the immunology of primary **lung cancer**.
Olsen GN, Gangemi JD.
Chest. 1985 May;87(5):677-83. Review.
PMID: 3886317

458. Usefulness of tumor markers in serum and **bronchoalveolar lavage** of patients undergoing fiberoptic bronchoscopy.
Goldstein N, Lippmann ML, Goldberg SK, Fein AM, Shapiro B, Leon SA.
Am Rev Respir Dis. 1985 Jul;132(1):60-4.
PMID: 4014874

Carcinoembryonic antigen in **bronchoalveolar lavage fluid**.
Lemarie C, Lavandier M, Renoux M, Renoux G.
N Engl J Med. 1980 Sep 4;303(10):586-7. No abstract available.
PMID: 7402229

Table 2 Diagnostic sensitivity of all the procedures

Procedures	Number of patients	Diagnosis achieved (%)
Bronchoalveolar lavage	30	14 (46.7)
Transbronchial biopsy	30	5 (16.7)
Postbronchoscopic sputum	26	2 (7.7)
All three procedures	30	15 (50)

Wongsurakiat et al. Respirology. 1998:3;131-137.

Research trends using BALF in lung cancer

▶ Kras

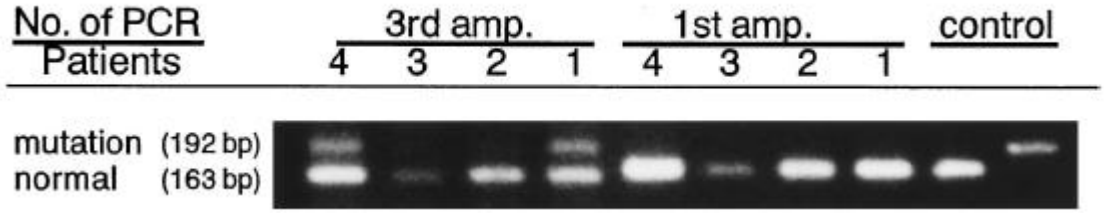


Table 3 Prevalence of K-*ras* codon 12 mutations in small pulmonary lesions by diagnosis

Detection of k-*ras* mutation in lung cancer was significantly higher than that in nonmalignancy ($P = 0.012$).

Diagnosis	No. of cases	
	Mutation (%)	Normal (%)
Lung cancer[a]	15 (75)	5 (25)
Nonmalignancy	4[b] (31)	9 (69)

[a] Adenocarcinoma (20).
[b] Focal fibrosis (3), pneumonia (1).

Oshita F et al. Clin Cancer Res. 1999 Mar;5(3):617-20.

▶ EGFR

No.	Tissue biopsy	Bronchoalveolar lavage			
		PNA PCR	PANAMutyper®	Site	DNA concentrations, ng/μL
1	p.E746_A750del	WT	WT	Rt. upper lobe	15.08
2	p.E746_A750del	E19del	–	Lt. upper lobe	10.64
3	p.E746_A750del	E19del	–	Lt. upper lobe	10.77
4	E19del	E19del	–	Lt. upper lobe	11.11
5	E19del	E19del	–	Rt. upper lobe	10.03
6	p.L858R	p.L858R	–	Rt. lower lobe	11.57
7	p.L858R	p.L858R	–	Rt. upper lobe	13.01
8	p.L858R	p.L858R	–	Rt. upper lobe	5.49
9	p.L858R	WT	p.L858R	Lt. lower lobe	9.43
10	p.L858R	p.L858R	–	Lt. lower lobe	8.53
11	p.L858R and p.T790M	p.L858R and p.T790M	–	Lt. lower lobe	11.98
12	E19del	WT	E19del	Rt. upper lobe	13.35
13	WT	WT	–	Lt. lower lobe	14.92
14	WT	WT	–	Lingular division	13.60
15	WT	WT	–	Rt. lower lobe	12.32
16	WT	WT	–	Rt. upper lobe	9.67
17	WT	WT	–	Lt. lower lobe	11.56
18	WT	WT	–	Rt. upper lobe	11.11
19	WT	WT	–	Rt. lower lobe	11.66
20	WT	WT	–	Rt. upper lobe	10.79

Park S et al. Clin Chem Lab Med. 2017 Feb 14.

BAL proteomics

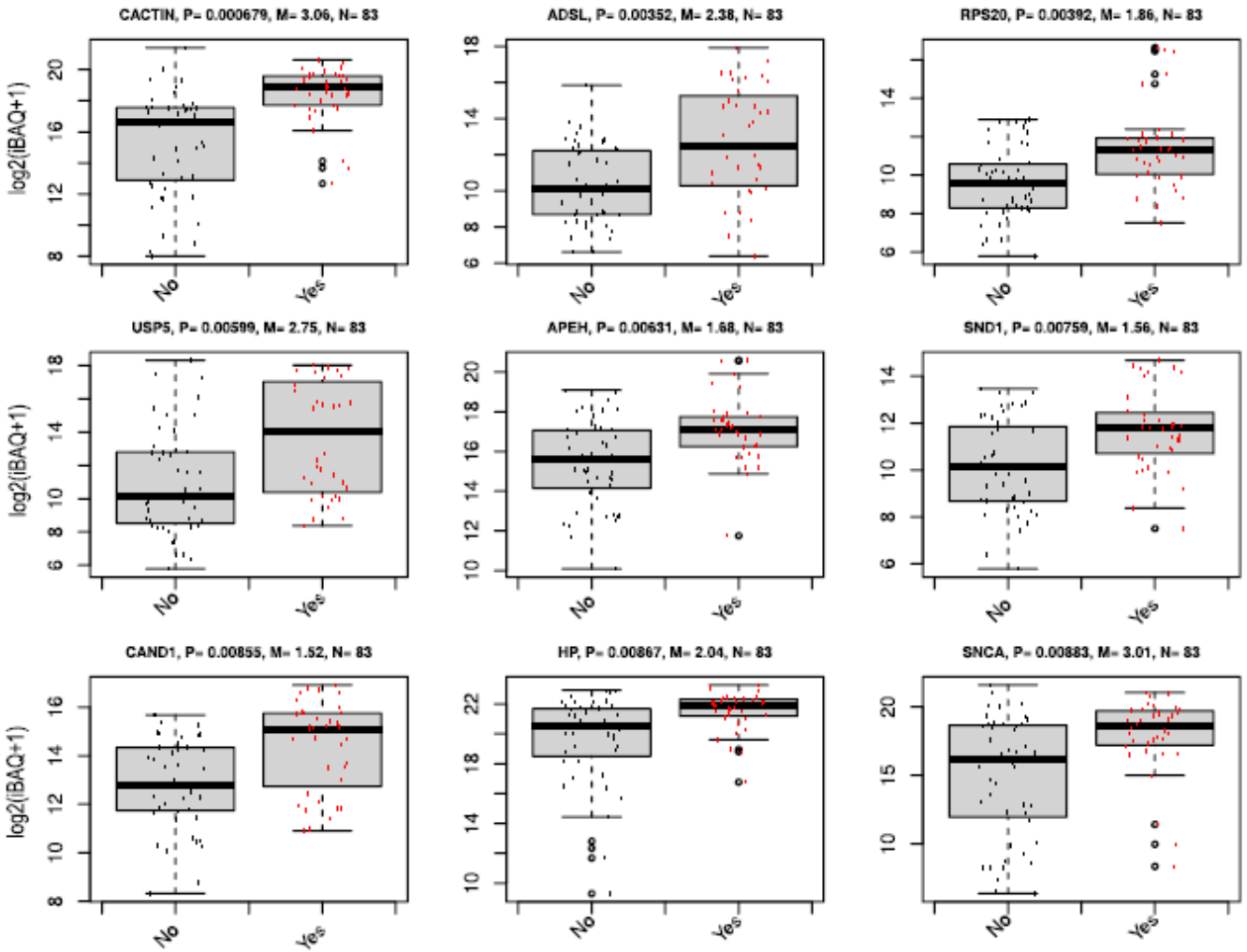


Figure 3. Boxplot of iBAQ expression values for the nine most significant regulated proteins between lung cancer cases and non cancer controls.

Carvalho AS et al. Sci Rep. 2017;7:42190.

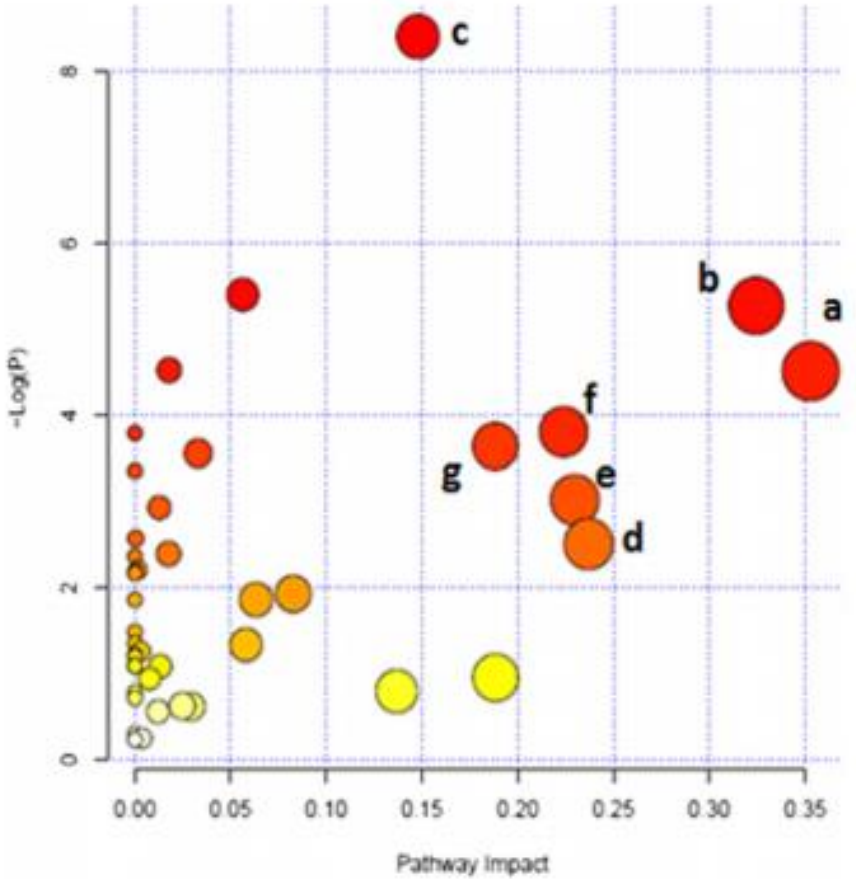


Fig. 5. Overview of the most important metabolomic changes observed in BALF from LC patients. a: D-Glutamine and D-glutamate metabolism, b: Glycine, serine and threonine metabolism, c: Glycerophospholipid metabolism, d: Pyruvate metabolism, e: Alanine, aspartate and glutamate, f: Galactose metabolism, g: Arginine and proline metabolism. *P* value is the p calculated from the enrichment analysis and Impact is the pathway impact value calculated from pathway topology analysis.

Callejón-Leblic B et al. J Proteomics. 2016 ;145:197-206.

Considerations (1)

- Technique
  - Keep suction pressure below 100 mm Hg and to avoid visible airway collapse
    - Low-pressure wall suction
    - Handheld syringe
    - Visual examination of the airway should allow monitoring for airway collapse during the aspiration process
  - At least 100 mL of instilled fluid
  - Target area

Video Article

Bronchoalveolar Lavage (BAL) for Research; Obtaining Adequate Sample Yield

URL: http://www.jove.com/video/4345

Andrea M. Collins[1,2], Jamie Rylance[3], Daniel G. Wootton[4], Angela D. Wright[3,5], Adam K. A. Wright[1,3], Duncan G. Fullerton[3,6], Stephen B. Gordon[3]

3. The Bronchoscope is Inserted and Positioned

1. Intubation is usually via the nose. If this is not possible due to nasal polyps, inflamed turbinates, or any discomfort then the subject is intubated via the mouth (a mouth-guard is used to prevent damage to the scope or the subject's teeth).
2. Topical anesthesia at the larynx is completed using 4% lidocaine, usually a total of 4-6 ml is used. Commonly this causes coughing on instillation.
3. The vocal cords are passed, and further mucosal anesthesia using 2 ml aliquots of 2% lidocaine at the carina, at the division of the right lower lobe (RLL) and right middle lobe (RML) and at the RML entrance.
4. The bronchoscope is positioned within the RML, ideally in the medial segment, in a position where it is distal enough to be in a secure position but not too distal so that the airway collapses when suction is applied ('Wink test' using the suction button).
5. Good positioning is indicated during the wink test by a bronchoscope that can be fully maintained in position by the bronchoscopist and an airway that does not fully close immediately on gentle suction.

4. The BAL is Performed

1. Four 60 ml syringes are prefilled with warmed normal saline - 60 ml, 50 ml, 50 ml, and 40 ml in successive syringes. When the subject and bronchoscopist are ready the first syringe of saline is instilled by the bronchoscopy assistant whilst the bronchoscopist maintains the position in the RML.
2. Gentle hand suction is then performed by the assistant using the same port and 50 ml syringe.
3. This procedure is then repeated a further 3x, with a maximal volume of 200 ml used in our specific technique.
4. The retrieved BAL fluid is expelled gently into labelled containers already held on melting ice. Glass containers may be presiliconized in order to inhibit cell attachment and maximize cell return.
5. The BAL fluid appears hazy against the light with surface soap bubbles formed by surfactants.
6. The bronchoscope is slowly fully withdrawn.
7. The BAL fluid is transported without delay on ice for immediate processing.

Bronchoalveolar Lavage (BAL) for Research; Obtaining Adequate Sample Yield

Andrea M. Collins[1,2], Jamie Rylance[3], Daniel G. Wootton[4], Angela D. Wright[3,5], Adam K. A. Wright[1,3], Duncan G. Fullerton[3,6], Stephen B. Gordon[3]

[1]Biomedical Research Centre in Microbial Diseases, **National Institute for Health Research**, [2]Respiratory Infection Group, **Royal Liverpool and Broadgreen University Hospital Trust**, [3]Respiratory Infection Group, **Liverpool School of Tropical Medicine**, [4]Institute of Infection and Global Health, **University of Liverpool**, [5]Comprehensive Local Research Network, **Royal Liverpool and Broadgreen University Hospital Trust**, [6]Department of Respiratory Research, **University Hospital Aintree**

Related Videos

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Published 4/13/2010

Translate text to:

Summary

Considerations (2)

- Handling of Aspirated Fluid
  - Recovered lavage fluid should be pooled into a single container (siliconized glass or noncell adherent plastic), well mixed, and the total volume measured
  - Transported to the laboratory on ice but can be stored or transported at room temperature if processing will occur in less than 1 hour

    (The cells remain viable in BAL fluid for up to 4 hours when stored at 25˚C)
  - An aliquot of the fluid and cells should be stored in case future testing is required
  - The aspirated fluid can contain mucous material as well as the fluid itself
    - Some groups have used filtering though gauze to remove the mucous material
    - Cells in the BAL fluid may variably adhere to the gauze
  - Cell counts should probably be made on unfiltered, unwashed, and unconcentrated samples

Applications

- ►Technique
  - 100-150mL of saline was instilled
  - Low-pressure wall suction (<100 mm Hg)
  - Target area : Main lesion > Middle lobe
  - Inadequate recovered fluid → additional washing, retract wedged scope
- ►Handling
  - Filtering though gauze to remove the mucous material
  - Transport within 1 hour
  - Centrifugation x 1000g for 10 minutes
  - Aliquot into a 1 mL and keep at -80˚C

Summary - Bronchoalveolar lavage

- Minimally invasive procedure
- Important information about immunologic, inflammatory, and infectious processes
- Reflect parenchymal changes on lung biopsy (tumor/ tumor environment)
- Limitations
  - Not standardized techniques
  - Variation among independent patients
  - Dilution
  - Contamination
  - Handling specimen
- Needs for consistent protocol according to a substance to be detected


Thank You!