AIRWAY SAMPLING TECHNIQUES IN ICU

Dr. Sharad Prasad Dahal

AIRWAY SAMPLING IN ICU

Proximal

- ETA
- Induced/Sputum
- Oral wash
- Nasopharyngeal swab

Non bronchoscopic

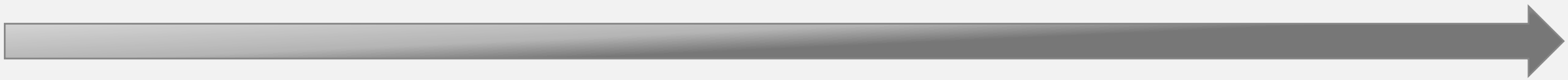
- Mini-BAL
- Blind PSB

Bronchoscopic

- BAL
- Protected specimen brush

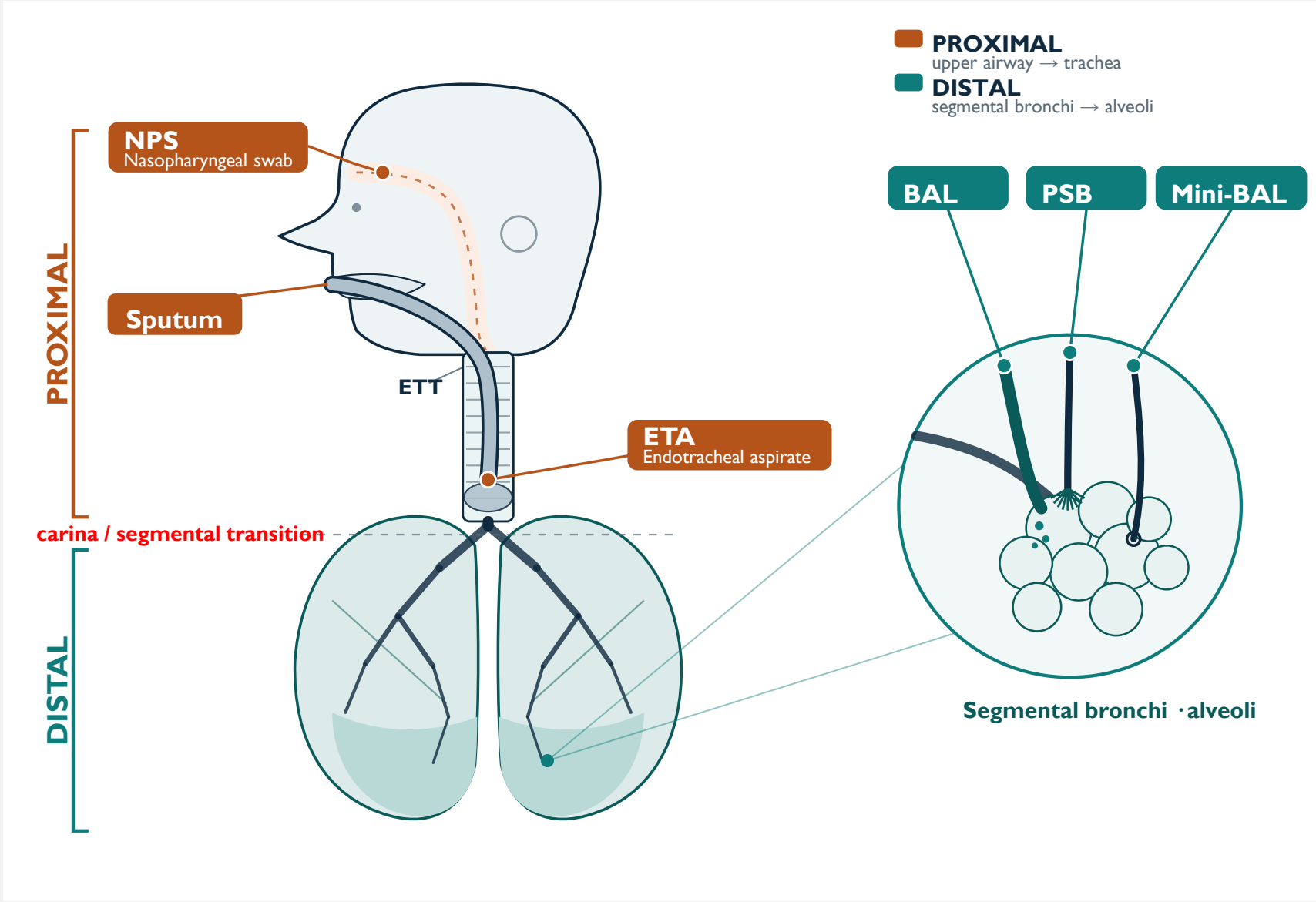
Tissue

- Lung Biopsy:
- Transbronchial
- Image guided
- Surgical



The sampling spectrum: proximal → distal

The sampling spectrum: Proximal → Distal



METHODS OF CULTURE

Qualitative cultures

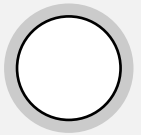
Specimen streaked on agar

- Reports **absence or presence** + **Organism identity and susceptibility**

Semiquantitative cultures

Specimen streaked across agar in successive quadrants and growth is graded by **how many quadrants grow**

- Reported as Light medium Heavy (1+ to 4+)
- Ordinal estimate
- ETA samples



Quantitative Cultures

Specimen is serially diluted and a measured volume plated , **colonies counted and reported as CFU/mL**

- Colonization vs infection can be separated objectively

Cochrane Database of Systematic reviews | Review - Intervention | New search | Free access

Quantitative versus qualitative cultures of respiratory secretions for clinical outcomes in patients with ventilator-associated pneumonia

Danilo Cortozi Berton, Andre C Kalil, Paulo José Zimermann Teixeira | Authors' declarations of interest

- 5064 articles: 3 RCT
- Compared Invasive method with quantitative cultures vs Non Invasive method with qualitative cultures

	All cause mortality	Inference
Qualitative vs Quantitative	25.4% vs 23.1%	RR: 0.91 (0.75-1.11)
Invasive-Non invasive	26.6% vs 24.7%	RR: 0.93 (0.78-1.11)

- No difference between all cause mortality, influence on Stewardship, rather than survival

If quantitative culture falls below the diagnostic threshold: antibiotic should be stopped

Berton et al, Cochrane reviews, 2014

PER-METHOD THRESHOLD

PSB

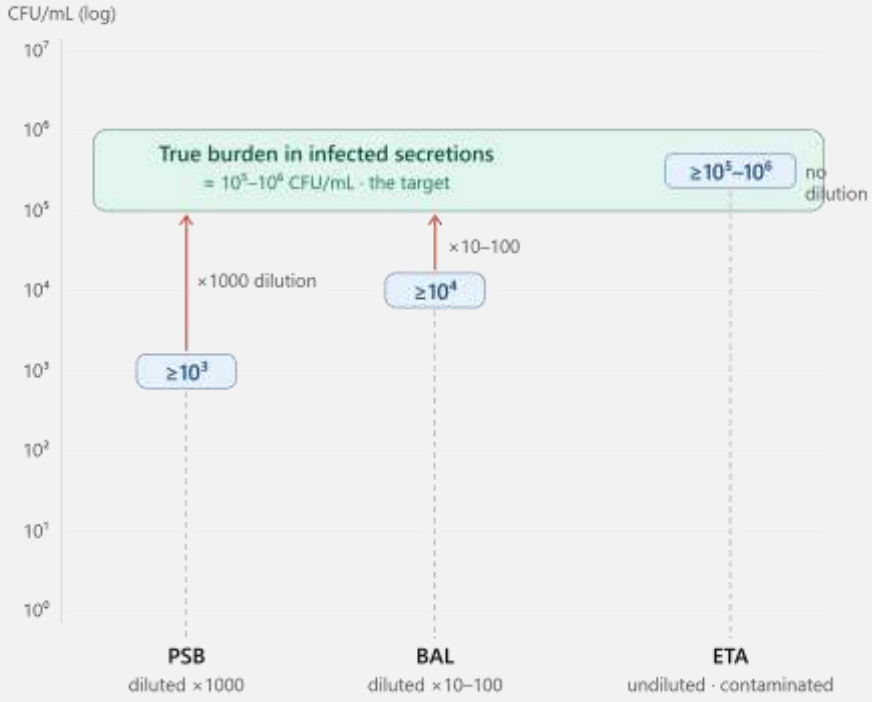
- Contains tiny fixed volume ~0.001ml of undiluted secretions
- Detected pathogens at ≥**10^3 CFU/mL** in histologically confirmed pneumonia [a]
- However reproducibility is poor : only ~17% paired sample crossed >10^3 CFU/mL[a]

BAL

- Instill and recovers large saline volume: Dilutes epithelial-lining fluid by 10-100 times
- ≥**10^4 CFU/mL** is s/o 10^5-10^6 in secretions [b]

ETA

- Contaminated by tube biofilm and airway colonization
- Needs highest bar to separate infection from colonization
- Lowest false-negative rates at ≥**10^5 CFU** [c]

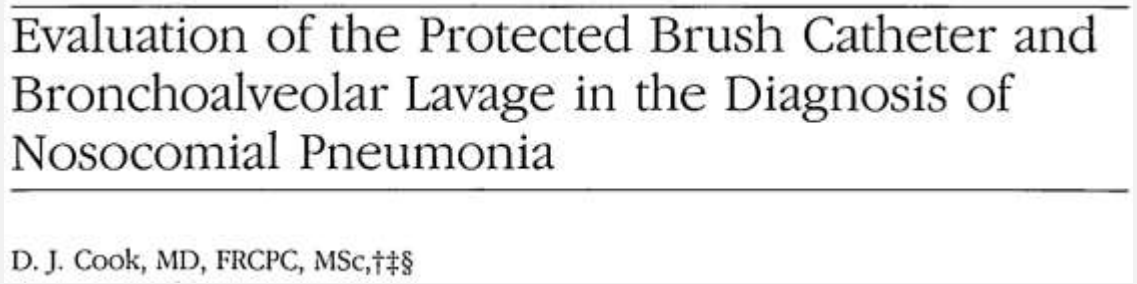


Same burden viewed through a different dilution.

a: Marquette et al, Chest,1993
b. Marquette et al, AJRCCM,1995
c. El-Ebiary et al. AJRCCM, 1995

Protected specimen brush (PSB)

- Telescoping double catheter: distal plug ejected in airway
- Inner brush takes ~0.001-0.01 mL distal, retraced before withdrawal
- Cutoff PSB ≥**10^3 CFU/mL**
- Reproducibility is poor : only ~17% paired sample crossed >10^3 CFU/mL



Evaluation of the Protected Brush Catheter and Bronchoalveolar Lavage in the Diagnosis of Nosocomial Pneumonia

D. J. Cook, MD, FRCPC, MSc,†‡§

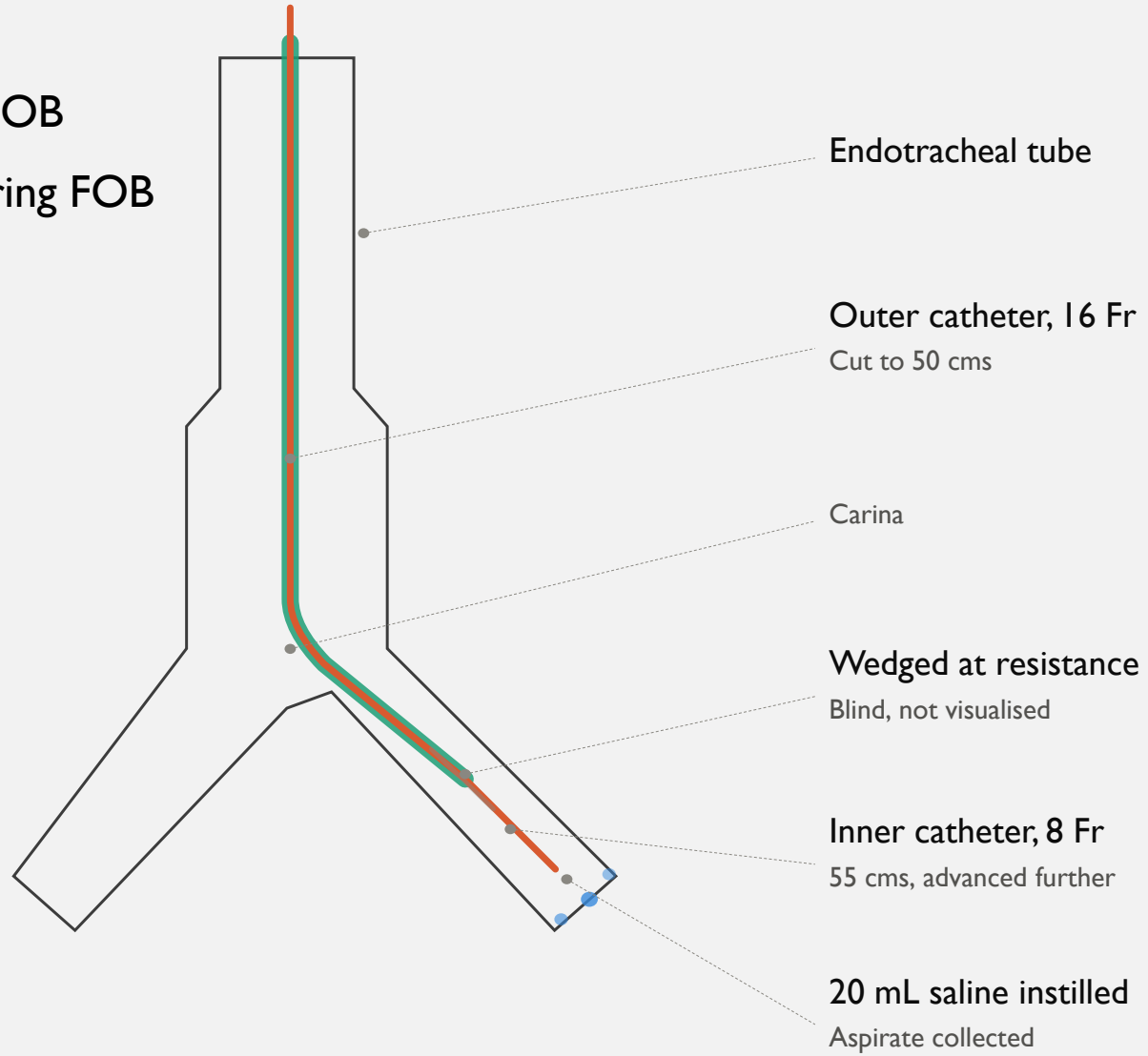
- 18 studies
- Reference test Composite clinical diagnosis : Positive Blood or pleural fluid cultures, rapid cavitation, or histological diagnosis
- Pooled sensitivity 89.9% and specificity 94.5%

Cook et al, JICM, 1991

Mini-BAL

- First used in 1989 for diagnosis of HAP
- Useful in low-resource setting with no access to FOB
- Mitigates inadvertent hypoxia, de-recruitment during FOB

- Is Mini-BAL equal to BAL for sampling in ICU?



Mini-BAL vs BAL: Accuracy

- 25 patients
- Immunocompromised excluded
- 4 respiratory sample evaluated in Each patient
  ETA→ mini-BAL→ Bronchoscopic brush→ BAL

Comparison of bronchoscopic and non-bronchoscopic techniques for diagnosis of ventilator associated pneumonia

G. C. Khilnani, T. K. Luqman Arafath, Vijay Hadda, Arti Kapil[1], Seema Sood[1], S. K. Sharma

- Definitive diagnosis of VAP taken as CPIS>6
- Yield of BAL: 64% and mini-BAL 68%

Table 2: Diagnostic value of various sampling techniques

Sampling techniques	Yield	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
ETA	52	55.6 (31.3–77.6)	71.4 (30.3–94.9)	83.3 (50.9–97.1)	38.5 (15.1–67.7)
NPBAL	68	83.3 (57.7–95.6)	71.4 (24.1–94.0)	88.2 (62.3–97.4)	62.5 (21.5–88.2)
BAL	64	77.8 (51.9–92.6)	71.8 (24.1–94.0)	87.3 (60.4–97.8)	55.5 (17.4–82.6)
B Brush	80	94.9 (70.6–99.7)	57.1 (13.4–86.1)	85 (61.1–96.0)	80 (21.9–98.7)

Table 3: Concordance among various microbiologic sampling techniques

Sampling techniques	Kappa coefficient	% Concordance	*P* value
NPBAL vs. B brush	0.694	88	0.001
BAL vs. B brush	0.423	76	0.04
NPBAL vs. BAL	0.56	80	0.01
ETA vs. NPBAL	0.351	68	0.064
ETA vs. BAL	0.272	64	0.161
ETA vs. B brush	0.426	72	0.009

- Predominant pathogens cultured were identical in 80% of mini-BAL vs BAL samples
- Recommends mini-BAL over ETA in diagnosis of VAP

<u>Limitations</u>

- CPIS score as reference standard not histological diagnosis
- Small cohort
- Timing of antimicrobial initiation and sampling not mentioned

Khilnani et al, JICM, 2023

Mini-BAL vs BAL: Accuracy

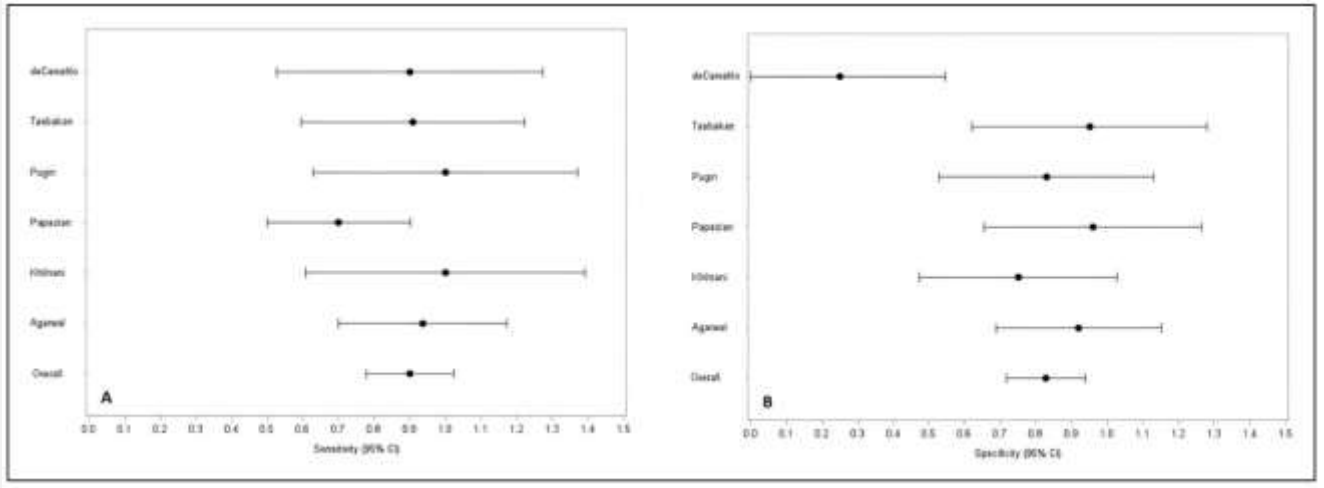
- Head to head design, 217 paired samples, 6 Studies
- Non-immunocompromised
- Each patient underwent both mini-BAL and BAL in succession (within 2 hrs)

Comparing the Accuracy of Mini-BAL to Bronchoscopic BAL in the Diagnosis of Pneumonia Among Ventilated Patients: A Systematic Literature Review

John Tepper, MD[1,2], Sean Johnson, MD[1,2], Connor Parker, MD[1,2], James Collins, MD[1], Laura Menard, MLS[3], and Laura Hinkle, MD[4]

Table 1. Study Characteristics.

Study/year	Number of pairs of resp. Samples	Average age	Female %	APACHE II score	Volume of saline instilled: bronchoscopic BAL (mL)	Volume of saline instilled: mini-BAL (mL)	Antibiotic days prior to BAL	Ventilator days prior to BAL
Agarwal et al/2020[14]	60	55.3	35%	20.3	20	20	NA	9.8
deCarvalho et al/2008[15]	22	59.4	59%	NA	100-150	20	21.0	5.6
Khilnani et al/2011[16]	25	55.6	32%	23.0	75	20	25.0	34.9
Papazian et al/1995[17]	38	63.6	34%	20.3	100	20	12.0	23.4
Pugin et al/1991[18]	40	47.8	25%	12.9	100-150	100	N/A	5.80
Tasbaskan et al/2011[19]	32	56.0	28%	23.6	120-150	20	NA	NA



- Mini-BAL was considered True positive only if identical organism grew at $>10^4$ CFU/ml
- Mini-BAL against bronchoscopic BAL:
  Sensitivity **0.90** (95% CI 0.78-1.00)
  Specificity **0.83** (0.72-0.94)

Caveat

- Small sample sizes (22-60 per study)
- Heterogenous saline volume (20-150ml)
- Large volume instillation noted with lower specificity (50%): high volume of instillation lets fluid migrate and pick up proximal flora
- No data on Viral or Mycosis

Tepper et al, JICM, 2023

Mini-BAL vs BAL: Immunocompromised

- 32 patients of SOT, HSCT, Malignancy , Use of CT, Steroid use (≥20 mg/day of pred for >2 weeks), PLHA
- ETA, mini-Bal and BAL was taken sequentially from all patients

Comparison of Bronchoalveolar Lavage and Mini-Bronchoalveolar Lavage in the Diagnosis of Pneumonia in Immunocompromised Patients

M. Sezai Tasbakan[a] Alev Gurgun[a] Ozen K. Basoglu[a] Pervin K. Ekren[a] Husnu Pullukcu[b] Feza Bacakoglu[a]

	BAL (n = 32)	Mini-BAL (n = 32)
Bacteriological results		
Acinetobacter baumannii	4	4
Streptococcus pneumoniae	2	2
MSSA	2	2
Cupriavidus pauculus	1	1
Enterococcus faecium	1	–
Pseudomonas aeruginosa	1	1
Yeast	3	2
No agent	18	20
Mycological results		
Candida spp.	11	12
Aspergillus spp.	–	1
No agent	21	19

- Strong correlations for bacterial (r=0.85) and fungal (r=0.82) detection between BAL & mini-BAL
- Poor correlation between ETA with BAL or Mini-BAL

		(r)
BAL bacteriology	mini-BAL bacteriology	0.850
BAL bacteriology	ETA bacteriology	0.477
Mini-BAL bacteriology	ETA bacteriology	0.430
BAL mycology	mini-BAL mycology	0.821
BAL mycology	ETA mycology	0.095
Mini-BAL mycology	ETA mycology	−0.176

Limitation

- Small sample size
- No mortality outcome comparison between mini-BAL and BAL

Tepper et al, JICM, 2023

Mini-BAL

- Is Mini-BAL equal to BAL for sampling in ICU?

YES

- Mini-BAL have similar yield and sensitivity to BAL
- Inadvertent effects of BAL can be mitigated by use of mini-BAL
- Mini-BAL is preferred method over ETA especially in immunocompromised

However

- No standardization of mini-BAL procedure
- Lack of large trials & Lack of gold standard: low quality of evidence
- Further exploratory trials in effect upon mortality and ICU stay is needed

PRE-ANALYTIC

- Early diagnosis and antibiotic stewardship improves outcomes in ICU
- Post antibiotic cultures under-detect organisms
- Specific diseases explored by this seminar
  1. VAP
  2. Viral CAP
  3. Invasive fungal pneumonia and GMI
  4. PcP
  5. CMV

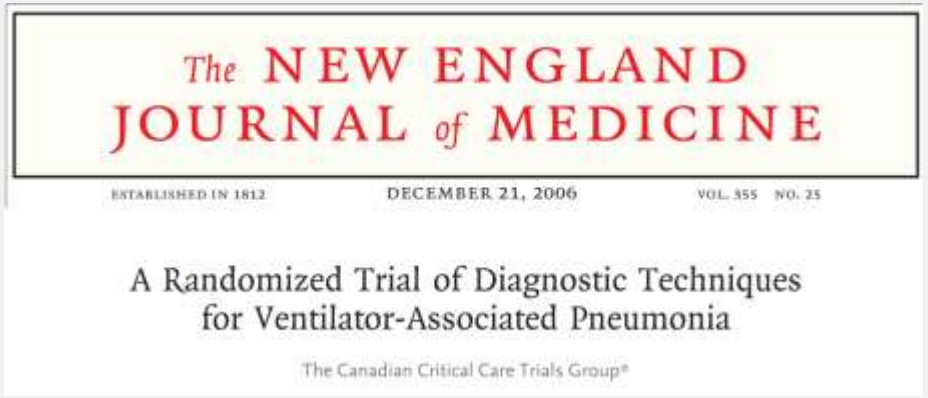
VAP

- Incidence ranges from 5 to 40% with all-cause mortality rate of 20-40%

Do cultures obtained from BAL has significant difference in clinical decision making compared to ETA?

HAP/VAP

- Multicentric RCT (28 ICUs):
  - Immunocompromised pt, pseudomonas and MRSA isolates were Excluded
- N=740 pt with suspected VAP
- Arm 1: BAL with quantitative culture
- Arm 2: ETA c.s
- Both received empirical antibiotics
- Primary Outcome: 28 days mortality rate
- Secondary Outcome: Survival in ICU & discharge from hospital, duration of mechanical ventilation, length of stay and response to microbiological Rx



- Majority were post cardiac surgery and trauma patients
- BAL yielded more positive cultures (59.7% vs. 51.9%, p = 0.03)

- 740 enrolled, Mean days from ICU stay to enrollment: 7.9 ± 5.2 days
  - Antibiotic therapy was standardized: 2 broad-spectrum antibiotics that are active against pseudomonas species

If c.s positive: De-escalate or switch to sensitive monotherapy
If c.s Negative: Treat pre-existing condition or Stop antibiotics

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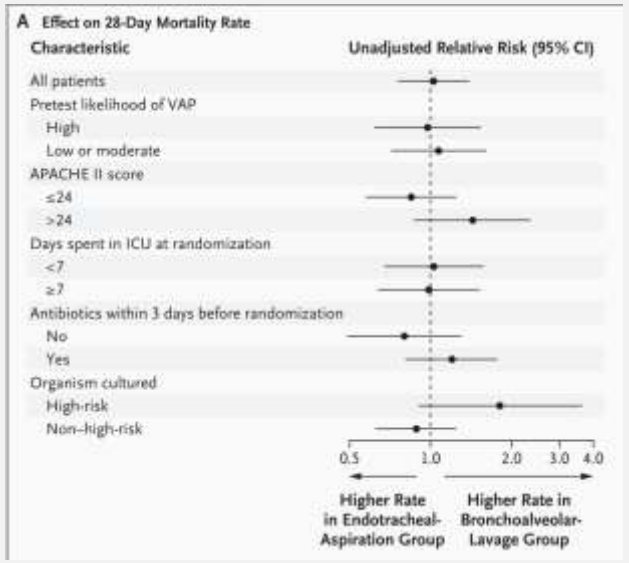
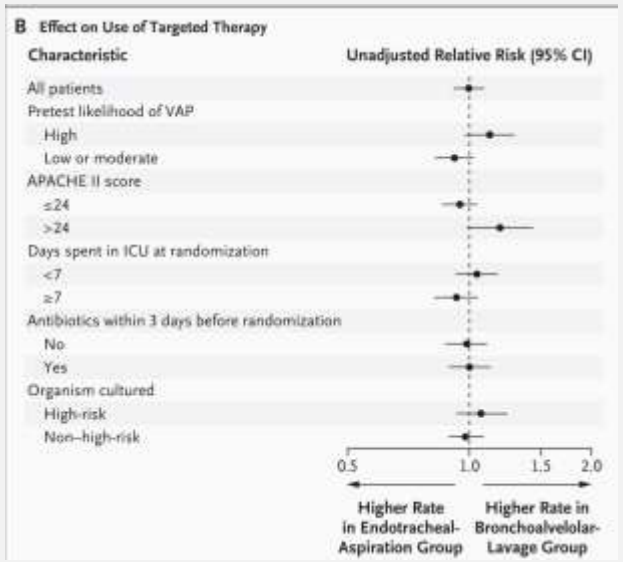
Heyland et al, NEJM, 2006

VAP

Table 5. Incidence of Targeted Therapy by Day 6.*

Patients	Endotracheal Aspiration	Bronchoalveolar Lavage	All	P Value
	no. of patients/total no. (%)			
All	279/374 (74.6)	271/365 (74.2)	550/739 (74.4)	0.90
Pretest likelihood of pneumonia				
High	108/162 (66.7)	132/177 (74.6)	240/339 (70.8)	0.11
Low or moderate	171/212 (80.7)	139/188 (73.9)	310/400 (77.5)	0.11
Positive culture				
All	148/194 (76.3)	172/218 (78.9)	320/412 (77.7)	0.63
Pretest likelihood of pneumonia				
High	68/89 (76.4)	96/120 (80.0)	164/209 (78.5)	0.71
Low or moderate	80/105 (76.2)	76/98 (77.6)	156/203 (76.8)	0.73
Negative culture				
All	131/180 (72.8)	99/147 (67.3)	230/327 (70.3)	0.28
Pretest likelihood of pneumonia				
High	40/73 (54.8)	36/57 (63.2)	76/130 (58.5)	0.28
Low or moderate	91/107 (85.0)	63/90 (70.0)	154/197 (78.2)	0.009





*Targeted therapy was defined as the discontinuation or modification of study antibiotics on the basis of the organisms cultured or the resumption of antibiotics to treat a preenrollment condition if the culture was negative

	ETA (n=374)	BAL (n=365)	RR* BAL/ETA	p-value*
Died within 14 days	49 (13.1%)	43 (11.8%)	0.88 (0.60-1.30)	0.53
Died within 28 days	69 (18.4%)	69 (18.9%)	1.01 (0.75-1.37)	0.94
Died in ICU	65 (17.4%)	58 (15.9%)	0.90 (0.65-1.25)	0.54
Died in Hospital	98 (26.2%)	84 (23.0%)	0.87 (0.67-1.12)	0.27

Did quantitative BAL tailored antibiotics more than ETA? → No (74.6% vs 74.2%)

No important differences in clinical outcomes or in the use of antibiotics between the groups undergoing either diagnostic test in the main analysis or in any prespecified subgroup analysis

Heyland et al, NEJM, 2006

Guidelines HAP/VAP

- **IDSA 2016:**
  - Suggest non-invasive sampling (ETA) with semiquantitative cultures than invasive sampling (BAL, PSB, mini-BAL)

- **ICS/NCCP 2012**
  - All sampling techniques (ETA, BAL or mini-BAL or PSB) are equally useful given cultures are quantitative &/or Semi-quantitative
  - Blind ETA sampling is easiest
  - Preferred method depending upon individual preferences, local expertise and cost

Guidelines

- **ERS 2017**
  - Suggest obtaining distal quantitative samples
    - ETA qualitative c.s. grows more organism than distal (biofilm of ET) and might be treated
    - Some observational studies showed significantly fewer superinfections in BAL group due to early stoppage of antibiotics owing to negative c.s.: Decrease in MDR superinfections
    - If taken before antibiotic initiation, negative predictive value of invasive samples are better than non invasive

  BUT,

    - Assumed invasive techniques widely available and feasible
    - No RCT compared qualitative and quantitative cultures of same bacteriological sample: always have confounding variable
    - Positive trial (French study, 2000) treated even commensals like coagulase negative staph isolated in proximal sampling : hence comparator have less antibiotic free day
    - Deleterious effect of invasive sampling especially in critically ill should be considered
    - Low quality of evidence

Fagon et. al, AIM, 2000

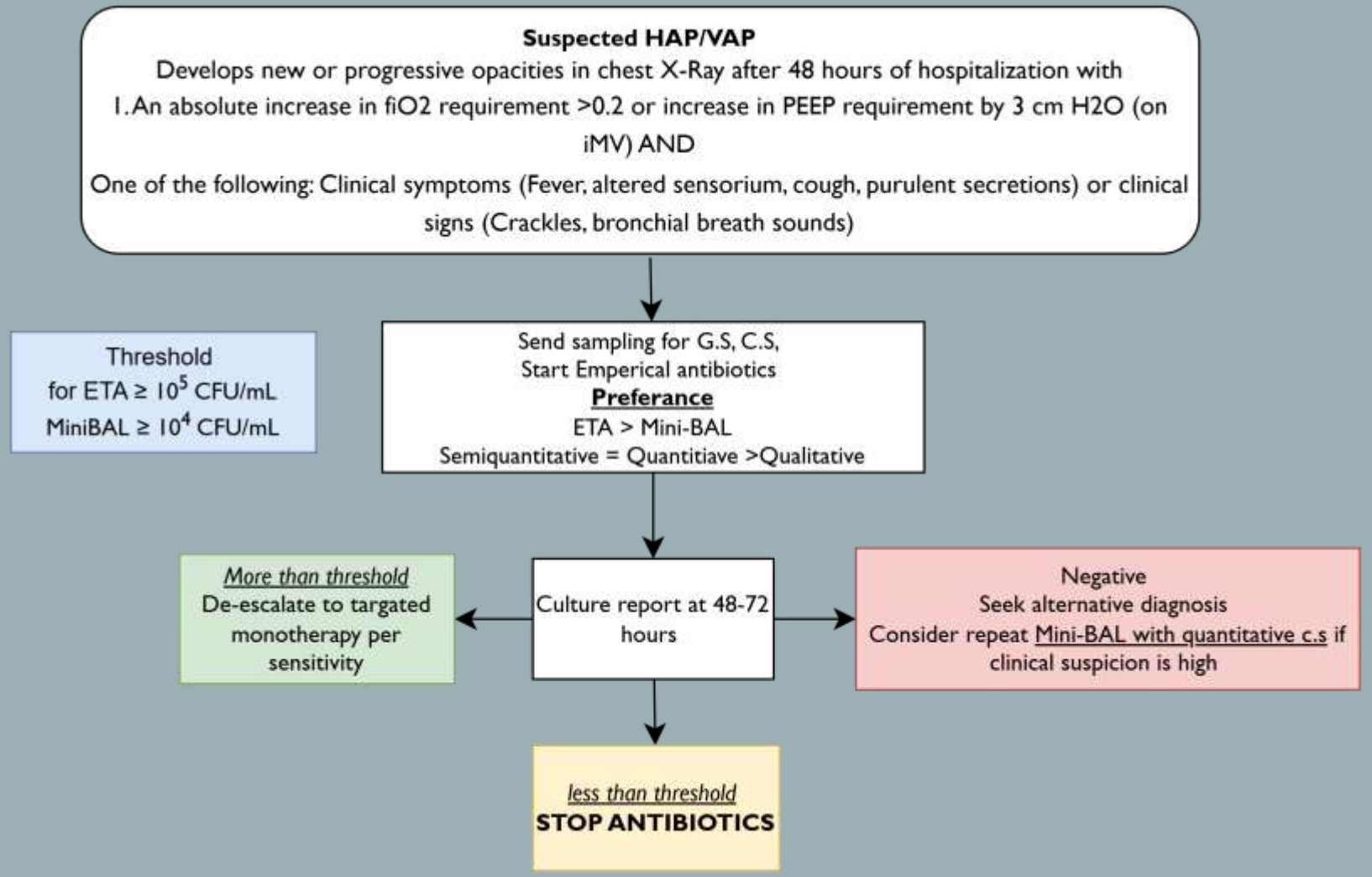
HAP/VAP

- Do cultures obtained from BAL has significant difference in clinical decision making compared to ETA?

NO

- Both methods influence on clinical outcome and antibiotic free days are comparable
- Protocolized empirical antibiotic initiation and early stopping on negative cultures is more important
- Method of culture : Quantitative or Semiquantitative is preferred
- Turnaround time of Cultures: either BAL or ETA: being same, might be late to influence survival

VAP

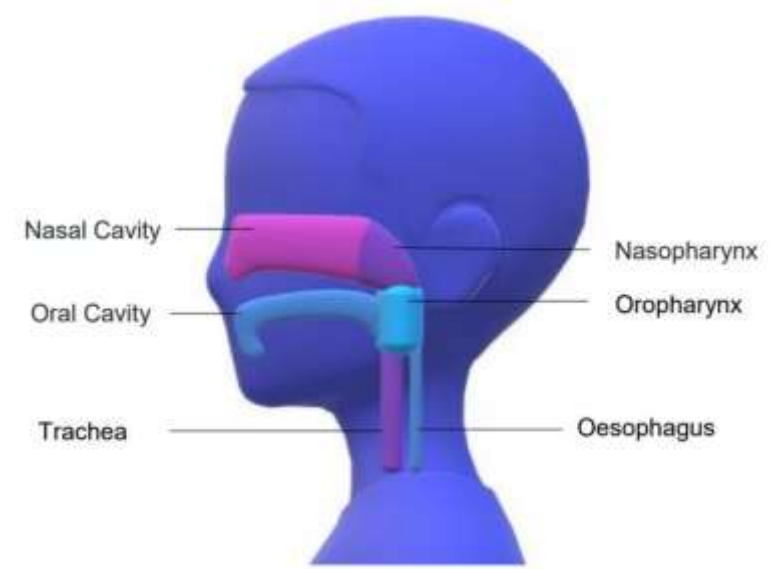

Suspected HAP/VAP
Develops new or progressive opacities in chest X-Ray after 48 hours of hospitalization with
1. An absolute increase in fiO2 requirement >0.2 or increase in PEEP requirement by 3 cm H2O (on iMV) AND
One of the following: Clinical symptoms (Fever, altered sensorium, cough, purulent secretions) or clinical signs (Crackles, bronchial breath sounds)
Threshold
for ETA ≥ 10^5 CFU/mL
MiniBAL ≥ 10^4 CFU/mL
Send sampling for G.S, C.S,
Start Emperical antibiotics
Preferance
ETA > Mini-BAL
Semiquantitative = Quantitiave >Qualitative
Culture report at 48-72 hours
More than threshold
De-escalate to targated monotherapy per sensitivity
Negative
Seek alternative diagnosis
Consider repeat Mini-BAL with quantitative c.s if clinical suspicion is high
less than threshold
STOP ANTIBIOTICS

Viral CAP

- RTPCR is reference method: IFA are fast but insensitive
- CT value are inversely proportional to viral load
- But detection ≠ disease: Prolonged shedding, co-detection and incidental carriage all confound
- In the ICU, the question is *where* to sample → NPS, ETA and BAL

Nasopharyngeal swabs

- Nasal sampling from middle turbinate
- Pharyngeal: Swab is inserted to a depth equal to the distance from the nostril to earlobe (Nasotragal length) or until nasopharynx is felt : depth of 14 cms

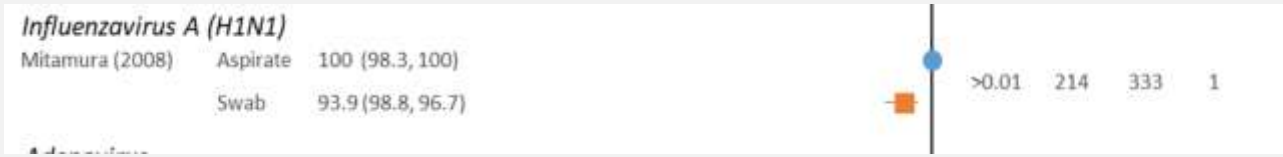


Systematic Review

Nasopharyngeal Swabs vs. Nasal Aspirates for Respiratory Virus Detection: A Systematic Review

Matthew F. Flynn [1,2,*], Martin Kelly [2] and James S. G. Dooley [1]

- 11 studies included; Used PCR (7), IFA (6) or both (1)
- NPS had sensitivity from 84-96% for RSV, Parainfluenza, metapneumovirus, influenza A+B
- NPS performs better in influenza A than nasal aspirate: Nasal aspirate reached 93% of swab sensitivity



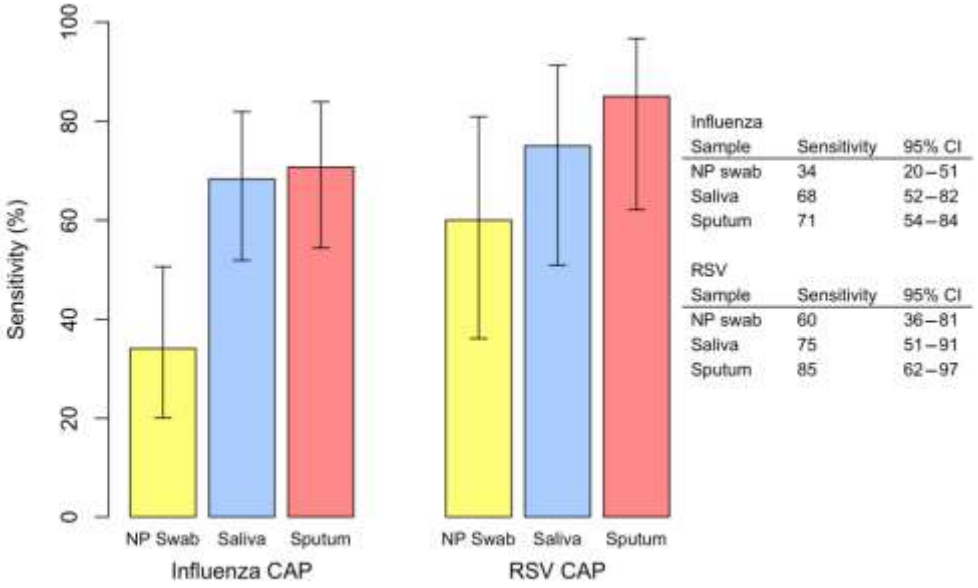
Flyyn et al, JICM, 1991

NP swabs: Performance in Viral CAP

Diagnosis of Community-Acquired Pneumonia Due to Influenza or Respiratory Syncytial Virus: Evaluation of RT-PCR Sensitivity in Nasopharyngeal, Saliva, and Sputum Samples

Julio Ramirez [1,2,*], Stephen Furmanek [1], Thomas Chandler [1], Ruth Carrico [1], Ashley Wilde [1], Alan Junkins [1] and Anupama Raghuram [1,2]

- Prospective study, 4 hospitals, 60 patients
- NP swabs, Saliva and sputum samples were obtained within 72 hrs
- 40 had Influenza, 19 had RSV



Influenza

Sample	Sensitivity	95% CI
NP swab	34	20–51
Saliva	68	52–82
Sputum	71	54–84

RSV

Sample	Sensitivity	95% CI
NP swab	60	36–81
Saliva	75	51–91
Sputum	85	62–97

Figure 2. Sensitivity by sample type of detection of influenza and RSV CAP.

- NP swab RT PCR detected only 34% of patients with Influenza CAP and 60% of patients with RSV CAP
- **Sputum sample** offered higher sensitivity for detecting either virus **with 85% sensitivity for RSV and 71% for Influenza**

For critically ill patients, Negative Nasopharyngeal swab alone should not be used to exclude Viral URTI

Ramirez et al, Pathogens, 2025

ETA in VTM for Viral CAP

Paired nasopharyngeal and deep lung testing for SARS-CoV2 reveals a viral gradient in critically ill patients: a multi-centre study.

Islam Hamed MSc[1*], Nesreen Shaban MSc[1*], Marwan Nassar MB BCh[1], Dilek Cayir MB BS,[1] Sam Love MRCP[1], Martin D Curran[2] PhD, FRCPath, Stephen Webb FFICM[3], Huina Yang FRCPath[3], Katherine Watson FRCPath[4], Anthony Rostron PhD, FFICM[4,5], Vilas Navapurkar FFICM[1], Razeen Mahroof FFCIM[1], Andrew Conway Morris PhD, FFICM[1,6]

- Retrospective, Multicentric evaluation
- 51, PCR confirmed COVID-19 patients
- Median days of sampling : 10 days (1-22) from symptom onset
- Paired sample: NPS or ETA sampling for RT PCR

- Median CT 19 in NP vs 19 in ETA : $p<0.0001$: Taken within 5 days of illness
- NP: 69% positivity
- Lung: 96% positivity

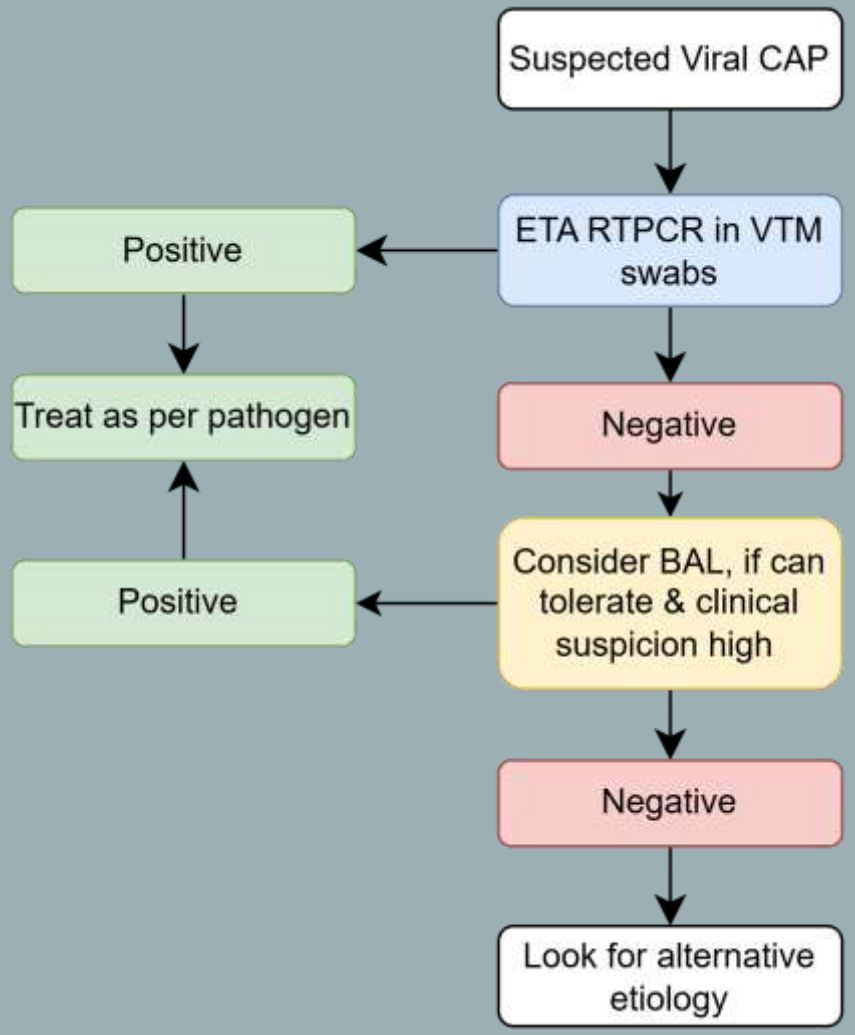
- As disease progress to critical illness: lower tracts viral load increases and upper tract shedding wanes
  - For critically ill patients, wherever feasible: **deeper respiratory samples should be obtained** in suspected severe COVID-19

Caveats

- Retrospective study
- Deeper samples may have been taken after negative NP
- Single disease so can't be extrapolated to other viral disease

Hamed et al., medRxiv 2020

Viral CAP


Suspected Viral CAP
ETA RTPCR in VTM swabs
Positive
Treat as per pathogen
Negative
Consider BAL, if can tolerate & clinical suspicion high
Positive
Negative
Look for alternative etiology

Invasive fungal pneumonia

- Direct microscopy; KOH/calcofluor stains,
  - for septate hyphae has low specificity for IPA (<30%),
  - high positive predictive value (>60%) in immunocompromised patients

- Rapid processing of samples is necessary to avoid bacterial and yeasts overgrowth

- Culture of high volume untreated sputum and BAL in specific media (SDA, BHI agar, PDA) is recommended

IPA: Smear & Fungal culture

- No head to head study on ETA vs BAL for smear

Fungal Culture

Systematic screening for COVID-19 associated invasive aspergillosis in ICU patients by culture and PCR on tracheal aspirate

Rebecca van Grootveld[1] | Judith van Paassen[2] | Mark G.J. de Boer[3] | Eric C.J. Claas[1]
Ed J. Kuijper[4] | Martha T. van der Beek[5] | LUMC-COVID-19 Research Group

- 63 COVID-19 patients on iMV
- ETA collected twice weekly for aspergillosis culture + PCR → BAL performed when ETA was positive

- Fungal culture from an **ETA missed no BAL-positive culture** (86% concordance)

But

- **Low culture yield (Only 6.3% were positive)**
- Cannot separate colonization from invasion: 3/15 ETA screen positives were BAL negative

Grootveld et. al, Mycoses. 2021

IPA: GMI

- Performance of ETA GMI
- Is Mini-BAL equal to BAL for GMI

ETA GMI: Performance

Accuracy of galactomannan testing on tracheal aspirates in COVID-19-associated pulmonary aspergillosis

Carla M. Roman-Montes[1,2] | Areli Martinez-Gamboa[1] | Paulette Diaz-Lomelí[1] | Axel Cervantes-Sanchez[1] | Andrea Rangel-Cordero[1] | Jose Sifuentes-Osornio[3] | Alfredo Ponce-de-Leon[1,2] | Maria F. Gonzalez-Lara[1,2]

- 144 consecutive COVID-19 patients enrolled
- CAPA was diagnosed in 14/144 (9.7%) patients BUT none met EORTC/MSG criteria
- CAPA had higher 28-day mortality (85% vs 40%)

Cut-off	0.8		1.0		1.5		2.0	
%	Sens	Spe	Sens	Spe	Sens	Spe	Sens	Spe
ETA:GMI	64.3	63	64.3	68.5	57.1	78.5	57.1	81.5

- No comparable BAL group

Dichtl et al Infection. 2023
Roman-Montes et al, Mycoses, 2020

Performance of galactomannan testing from endotracheal aspirate to guide bronchoalveolar lavage in the diagnosis of invasive aspergillosis

Karl Dichtl[1,2] · Rachel Barry[3] · Matthias W. A. Angstwurm[4] · Sebastian Suerbaum[1] · Johannes Wagener[3,5]

- Retrospective study
- 133 patients (All subsequent patients) in which both ETA and BAL was sent for GMI were studied

- 61 BAL samples were positive for GMI (> ODI)
- When EORTC?MSGERC criteria was applied: 4/61 were Proven IPA, 19/61 were Probable IPA
- Rest 38/61 were not categorizable due to absence of host factors

ETA GMI	Cutoff ≥0.50	Cutoff ≥1
Sensitivity	**92%**	**91%**
NPV		**98%**
Specificity	**72% (Cutoff 0.63)**	

- ETA GMI can avoid unnecessary BAL i.v.o high NPV
- **But proven IPA proportion was small**

Invasive fungal pneumonia

- Performance of ETA GMI
- Is Mini-BAL equal to BAL for GMI

POOR

- ETA GMI has promising NPV but has limited PPV and Specificity
- Large head to head comparison studies with BAL and serum GMI is necessary for validation

IPA in ICU

- Performance of ETA GMI
- Is Mini-BAL equal to BAL for GMI

Mini-BAL vs BAL: GMI

- 32 patients with haematological malignancies with compatible radiology
- 72% had severe neutropenia
- Underwent mini-Bal and BAL (from target lesion) for GMI along with serum GMI

Galactomannan-Antigen Testing from Non-Directed Bronchial Lavage for Rapid Detection of Invasive Pulmonary Aspergillosis in Critically Ill Patients: A Proof-of-Concept Study

Kathrin Rothe [1,2], Miriam Dibos [3], Stefanie J. Haschka [3], Roland M. Schmid [3], Dirk Busch [3], Sebastian Rasch [3] and Tobias Lahmer [3,*]

Table 2. Results of galactomannan-antigen testing from bronchoalveolar lavage fluid and tracheal secretion.

	Galactomannan from BAL:	Galactomannan from NBL:	*p*-Value
Mean galactomannan (ODI)	3.6 ± 2.2	4.3 ± 2.4	0.697
Probable invasive aspergillosis (*n*)	32		
Positive galactomannan result (*n*)	32		
Cultural microbiological evidence (*n*)	12 (all *A. fumigatus*)		

- 37.5% of BAL fluid grew A. fumigatus
- Culture not done in mini-BAL group
- **Comparable GMI values in both BAL and Mini-BAL**

But studies with proven IPA and large sample size are lacking

Rothe et al, Diagnostics 2023

IPA in ICU

- Performance of ETA GMI
- Is Mini-BAL equal to BAL for GMI

NO

- Even though mini-BAL & BAL GMI values are comparable: Studies are sparse and evidence quality is not strong
- Further adequately powered studies with large sample to assess mortality benefit are needed

PcP in ICU

- IFA of PcP tracks density of cysts/trophozoites in specimen : burden dependant
- Organism load of PcP is lower in non-HIV patients than HIV: performance of diagnostic test is higher in PLHA

PcP in ICU

- Observational, retrospective study from Spain
- Total 299 patients: 65 **Sputum** samples, 53 **ETA** and 181 **BAL** samples were analysed
- 48 patients (16%) were diagnosed with PcP

	With PJP *n* (%)	Without PJP *n* (%)	*p* Value
Patients	48	251	
Median age (years, IQR)	57 (49–69)	58 (45–69)	0.83
Sex (male)	31 (65)	163 (65)	0.96
Underlying disease			
Hematologic malignancy	10 (21)	53 (21)	0.965
HIV infection	12 (25)	41 (16)	0.150
Solid tumor	16 (33)	36 (14)	<0.01
Solid organ transplantation	1 (2)	24 (10)	0.09
Autoimmune disease	3 (6)	16 (6)	0.97
Steroid exposure	3 (6)	9 (4)	0.39
Other [a]	0	1 (0.4)	0.66
No immunosuppression	3 (6)	71 (28)	<0.01
Previous PJP prophylaxis	2 (4)	59 (24)	<0.01

Brief Report

Pneumocystis jirovecii Pneumonia Diagnostic Approach: Real-Life Experience in a Tertiary Centre

Cristina Veintimilla [1,2], Ana Álvarez-Uría [1,2], Pablo Martín-Rabadán [1,2,3], Maricela Valerio [1,2,4], Marina Machado [1,2], Belén Padilla [1,2], Roberto Alonso [1,2,4], Cristina Diez [1,2,5], Patricia Muñoz [1,2,3,4,*] and Mercedes Marín [1,2,3,4]

Table 3. Validation of PCR and IFA assays by type of respiratory sample.

	BALF % (95% CI)		Tracheal Aspirate % (95% CI)		Sputum % (95% CI)	
	IFA	PCR	IFA	PCR	IFA	PCR
Sensitivity	24 (11.5–43.4)	100 (86.7–100)	33.3 (9.7–70)	100 (61–100)	29.4 (13.3–53.1)	83.2 (65.7–96.7)
Specificity	100 (97.6–100)	99.4 (96.5–100)	100 (92.4–100)	100 (92.4–100)	100 (92.6–100)	91.7 (80.4–97)
PPV	100 (61–100)	96.2 (81.1–99.3)	100 (34.2–100)	100 (61–100)	100 (56.6–100)	78.9 (56.7–91.5)
NPV	89.1 (83.7–92.9)	100 (96.9–100)	92.2 (81.5–97)	100 (92.4–100)	80 (68.2–88.2)	95.7 (85.5–98.8)

Abbreviations: BALF, bronchoalveolar lavage fluid; CI, confidence interval; PPV, positive predictive value; NPV, negative predictive value.

- Real-time PCR largely equalizes the sampling-technique differences
  - PCR "rescues" the proximal sample that IFA would miss

Veintimilla et al, Fungi, 2023

PcP in ICU

- Prospective study, Solid tumour, SOT, DM, Hematologic malignancy
- 249 patients underwent BAL and ETA

Endotracheal aspirate as an alternative to bronchoalveolar lavage fluid for the diagnosis of *Pneumocystis* pneumonia by real-time polymerase chain reaction

Sang-Ho Choi [1, #], Sang-Bum Hong [2, #], Jin Won Huh [2], Heungsup Sung [3], Kyung-Hyun Do [4], Sang-Oh Lee [1], Chae-Man Lim [2], Younsuck Koh [2, *]

Performance of endotracheal aspirate real-time PCR for the diagnosis of *Pneumocystis jirovecii* pneumonia

	BAL positive	BAL negative	Sensitivity (95% CI)	Specificity (95% CI)	Accuracy (95% CI)	PPV[a] (95% CI)	NPV[a] (95% CI)	PLR (95% CI)	NLR (95% CI)
BAL fluid real-time PCR cutoff: Ct ≤ 40[b]									
Endotracheal aspirate positive ($n = 72$)	59	13	85.5% (75.0–92.8)	92.8% (88.0–96.1)	90.7% (86.4–94.0)	82.4% (73.3–88.8)	94.2% (90.1–96.7)	11.84 (6.95–20.17)	0.16 (0.09–0.28)
Endotracheal aspirate negative ($n = 177$)	10	167							
Total	69	180							

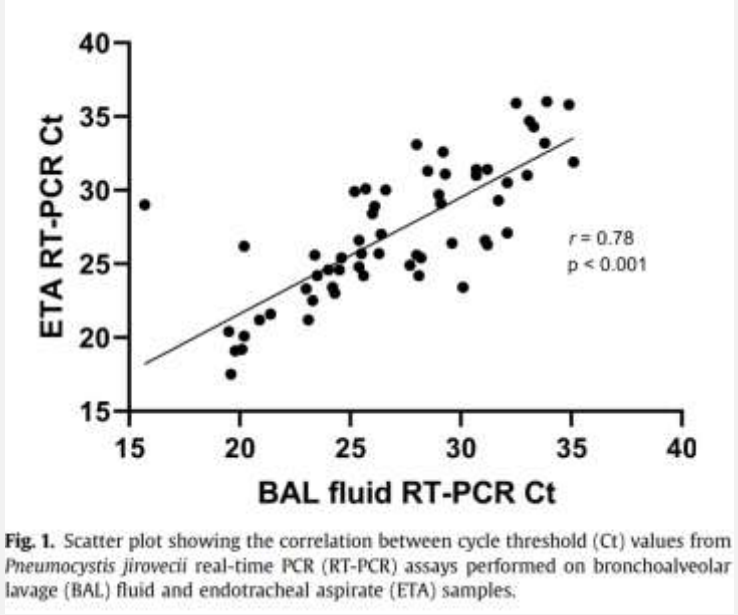


Fig. 1. Scatter plot showing the correlation between cycle threshold (Ct) values from *Pneumocystis jirovecii* real-time PCR (RT-PCR) assays performed on bronchoalveolar lavage (BAL) fluid and endotracheal aspirate (ETA) samples.

- High sensitivity and Strong negative predictive value
- High concordance between ETA PCR and BAL PCR

Choi et al, Clinical Microbiology and Infection, 2025

PcP in ICU

- ETA has high concordance with BAL when PCR is used for diagnosis
- As IFA is burden dependant: ETA for PcP IFA in PLHA cases : where burden is higher, might be used: however evidence is poor

PcP in ICU: Mini-BAL

- No comparative study or systematic reviews in use of mini-BAL in PcP diagnosis till date

PcP: Other techniques

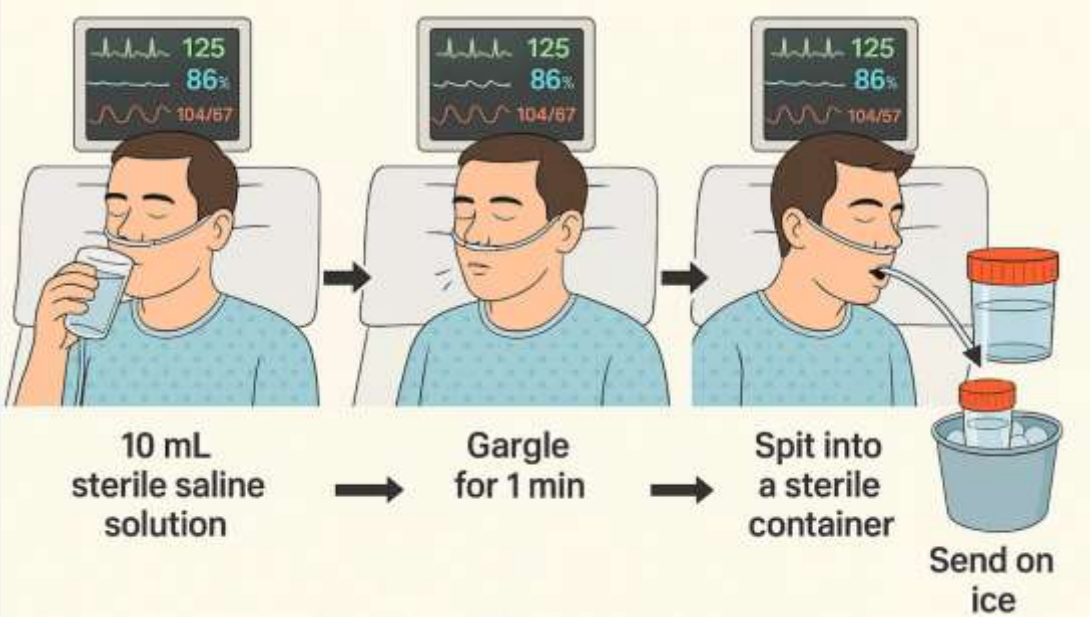
Systematic Review

Pneumocystis jirovecii Pneumonia Diagnosis with Oropharyngeal Wash PCR in Immunocompromised Patients—A Systematic Review

Vasco Salgado Costa [1,2,*], José Pedro Cidade [1,2], Inês Medeiros [1], Pedro Fidalgo [1], Hugo Moreira [1], Teresa Miranda [1] and Pedro Póvoa [1,2,3,4]

- 12 studies reviewed between 1996 and 2016
- Included immunocompromised adult, most were HIV positive (except 2 studies)
- BAL was reference standard
- 10mls saline gargling for 5 to 120 secs (Most common 60 second gargle)→ sent for PCR

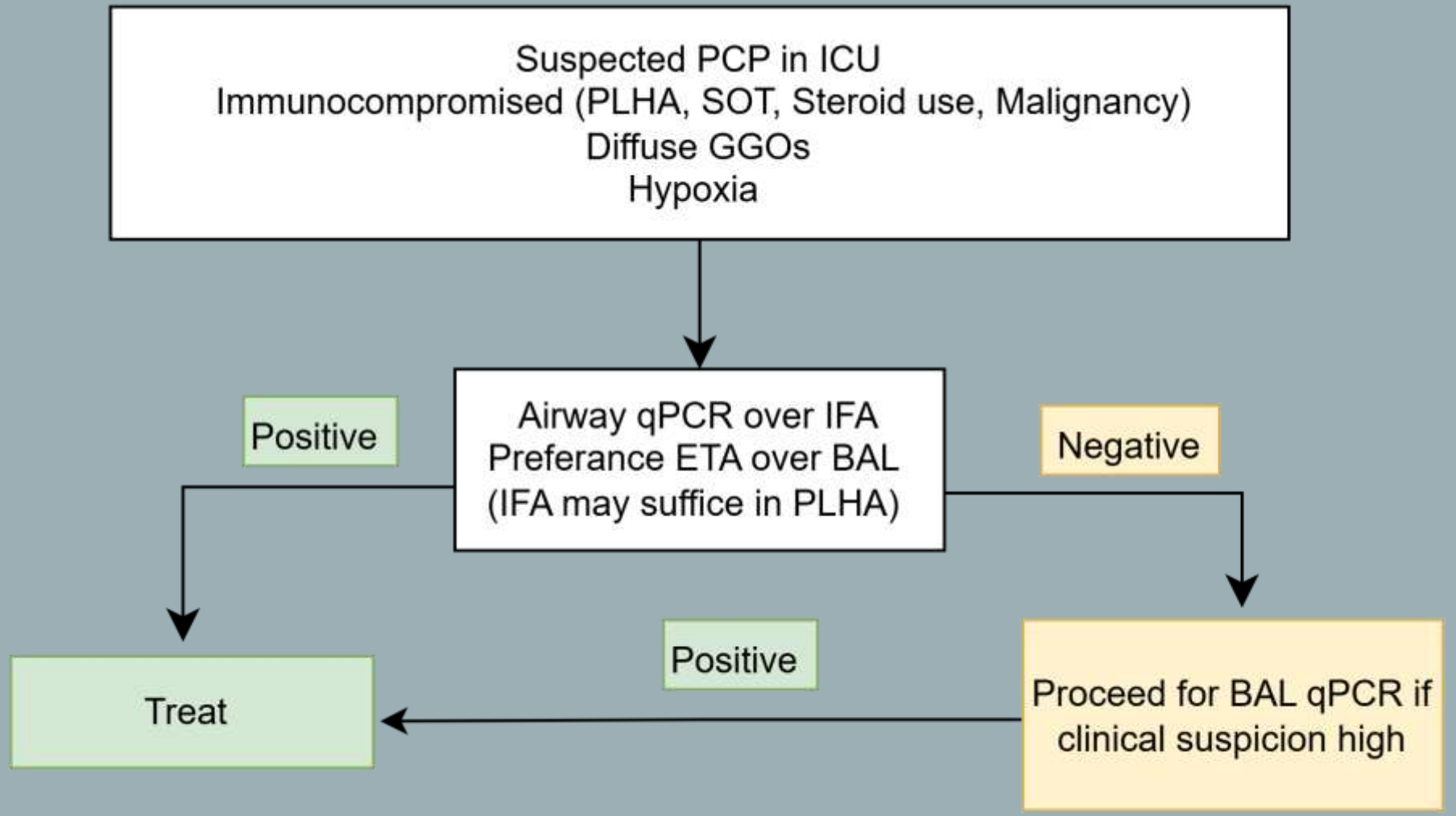
- Pooled sensitivity 68%, Specificity 92% and Pooled false positive was 8%



- Oral wash PCR can be used when sampling is not feasible in moderate to high risk patients
- **Negative OW PCR→ In stable non high risk : may rule out infection**
- **No standardized procedure:** poor sensitivity even on high burden cases of PLHA

Costa et al, J. Clin. Med. 2025

PcP


Suspected PCP in ICU
Immunocompromised (PLHA, SOT, Steroid use, Malignancy)
Diffuse GGOs
Hypoxia
Airway qPCR over IFA
Preferance ETA over BAL
(IFA may suffice in PLHA)
Positive
Negative
Treat
Positive
Proceed for BAL qPCR if clinical suspicion high

CMV pneumonitis

Proven	Clinical symptoms / signs of pneumonia (e.g., hypoxia, imaging findings), and CMV documented in lung tissue by virus isolation and histopathology or IHC
Probable	Clinical symptoms/signs + CMV in BAL fluid by viral isolation or CMV DNA quantitation - the threshold for CMV DNA load in BAL fluid is not established
Possible	Clinical + radiologic features, CMV DNA detection in BAL is borderline / lower, where there are co-pathogens present ,making attribution uncertain

Treat	Treat empirically	Do Not Treat
▪ Proven CMV pneumonitis (tissue/IHC) ▪ Probable CMV pneumonitis: hypoxemia + compatible CT + BAL CMV high (>500 IU/mL) and no alternative cause ▪ Organ dysfunction (Colitis, Retinitis, Organomegaly not explained otherwise) + High CMV titres (Cutoff >500IU/mL) in blood/serum	• Severe respiratory failure + high BAL viral load or progressive disease despite treatment of copathogens • CMV reactivation in ICU {associates with worse outcomes (OR ≈2.5 for mortality)}	• low BAL DNAemia without clinical syndrome

Ljungman et al, J. Clin Infect Dis2017

CMV: BAL

Cytomegalovirus (CMV) DNA Quantitation in Bronchoalveolar Lavage Fluid From Hematopoietic Stem Cell Transplant Recipients With CMV Pneumonia

Michael Boeckh,[1,2,3] Terry Stevens-Ayers,[1] Giovanna Travi,[1,a] Mei-Li Huang,[1,b] Guang-Shing Cheng,[2,3] Hu Xie,[2] Wendy Leisenring,[2,4] Veronique Erard,[1,a] Sachiko Seo,[1,a] Louise Kimball,[1] Lawrence Corey,[1,2,5] Steven A Pergam,[1,2,3] and Keith R. Jerome[4,5]

- 132 HCT patients vs 139 controls underwent BAL
- Median days from transplant was 45 days

- >500 IU/mL in BAL discriminates CMV pneumonia from pulmonary shedding
- Co-pathogen and haemorrhage did not affect the value
- In high prevalence situation anti-CMV treatment Naïve population **200 or 100 IU//mL** can be used

Diagnosis of cytomegalovirus pneumonia by quantitative polymerase chain reaction using bronchial washing fluid from patients with hematologic malignancies

Hwa Young Lee[1,*], Chin Kook Rhee[1,*], Joon Young Choi[1], Hea Yon Lee[1], Jong Wook Lee[2,3] and Dong Gun Lee[3,4,5]

- 565 haematological malignancies patients underwent BAL → 101 had positive CMV PCR, 24 were CMV pneumonia (Rest shedding or excluded)
- Conversion: One copy of CMV DNA using the AccuPower CMV Quantitative PCR test was equivalent to 7.3 International Units (IU)
- CMV pneumonia patients had 6 fold higher mean blood CMV loads vs No CMV pneumonia

- **Cut-off value 28,774 copie**s i.e. **210,000 IU/mL**: Sensitivity 75%, Specificity 88.6%, NPV 99.8%

Boeckh et al, JID 2017
Lee et al, Oncotarget, 2017

CMV: Addressing the Gap

NO DEFINITIVE STANDARD

- Problems with BAL CMV PCR: Heterogenous sample: analysed sample doesn't represent whole sample due to dilution unlike blood
- Difference in sampling technique : BAL done with single aliquot and aspiration vs Wedging and serial instillation of small volume fluid till 20ml is aspirated
- Different platform use: **Boeckh's** in-house gB/pp65 real-time PCR reporting directly in IU vs **Lee's** Bioneer AccuPower reporting copies, then multiplied by a lab-derived 7.3 IU/copy factor vs **PGIMER** Nuclisensse Easymag extraction f/b TRUPCR CMV QT kit
- Heterogenous population

Table 2. DNA Levels in Bronchoalveolar Lavage Fluid (IU/mL) for Diagnosis of Probable or Unlikely Cytomegalovirus Pneumonia

Patient Category	Probable	Unlikely
Allogeneic HCT patients[a]	$\geq 10^3$[a]	$< 5 \times 10^2$
Autologous HCT patients CAR T cell-treated patients	Cannot be defined	$< 5 \times 10^2$
Solid organ transplant patients except lung transplant patients isolated or combined	Cannot be defined	$< 5 \times 10^2$
Lung transplant patients	Cannot be defined	Cannot be defined

The Fourth International Consensus Guidelines on the Management of Cytomegalovirus in Solid Organ Transplantation

Camille N. Kotton, MD,[1] Deepali Kumar, MD,[2] Oriol Manuel, MD,[3] Sunwen Chou, MD,[4] Randall T. Hayden, MD,[5] Lara Danziger-Isakov, MD, MPH,[6] Anders Asberg, PhD,[7] Helio Tedesco-Silva, MD,[8] and Atul Humar, MD[2]; on behalf of The Transplantation Society International CMV Consensus Group*

Kotton et al, Transplantation, 2025

CMV: ETA

Virological and Immunological Features of Active Cytomegalovirus Infection in Nonimmunosuppressed Patients in a Surgical and Trauma Intensive Care Unit

Marifina Chilet, Gerardo Aguilar, Isabel Benet, Javier Belda, Nuria Tormo, José Antonio Carbonell, María Ángeles Clari, Elisa Costa, David Navarro

- Prospective study , 53 patients admitted in Post surgical ICU
- Paired sampling (Plasma and ETA) virological monitoring done once a week
- Reactivation occurred in 40% of patient
- 23% of CMV DNA was detectable only in ETA, never in Plasma
- Peak CMV DNA levels were significantly higher in ETA than in plasma

- **Sensitivity and specificity is poor**

- CMV Infections compartmentalized to respiratory tract can be detected by ETA PCR, before, or even in the complete absence of, positive blood PCR.

BUT

- Cannot differentiate between shedding & true disease
- No RCT , no diagnostic accuracy study

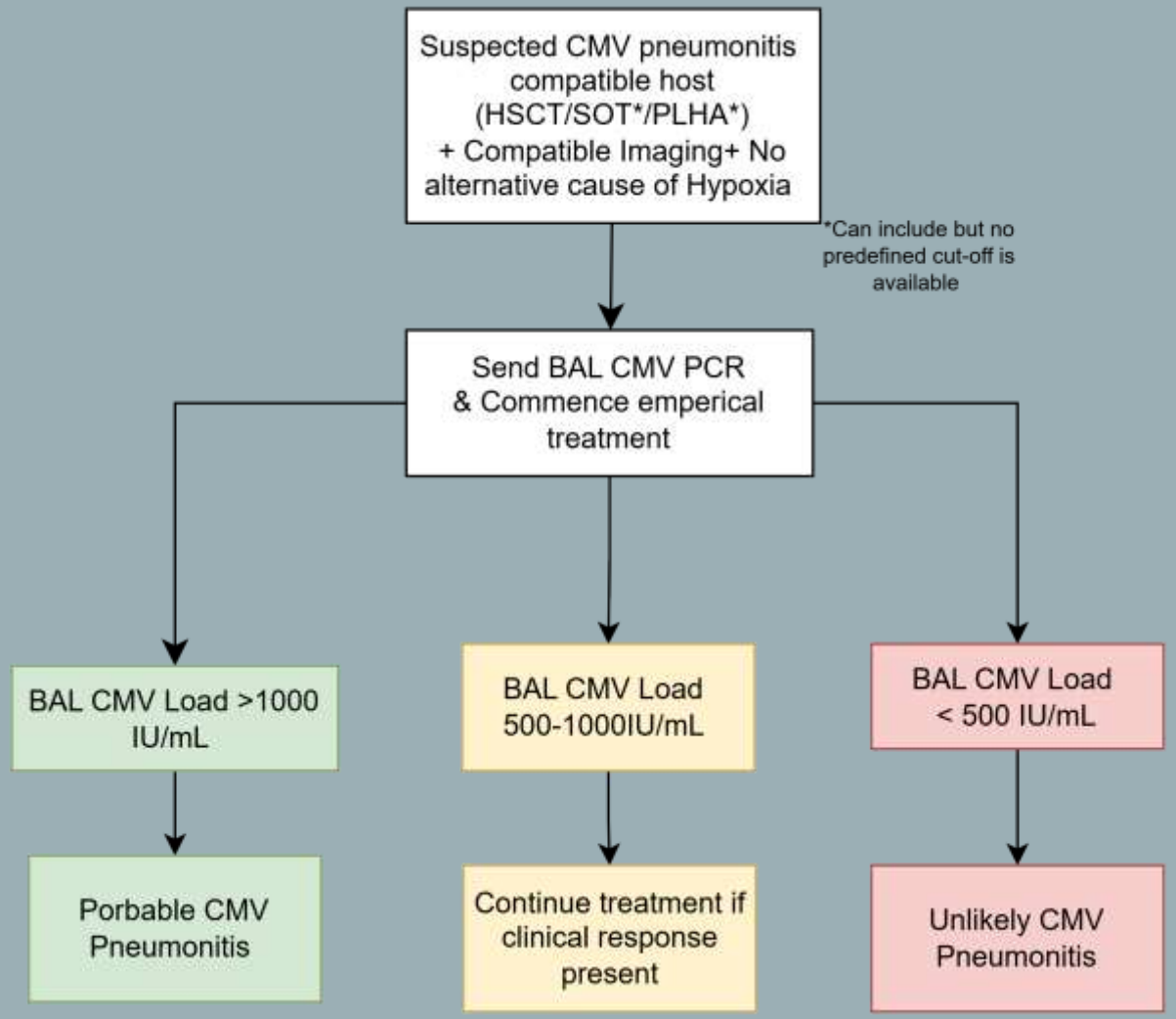
Chilet et al, hal , 2011

CMV: ETA

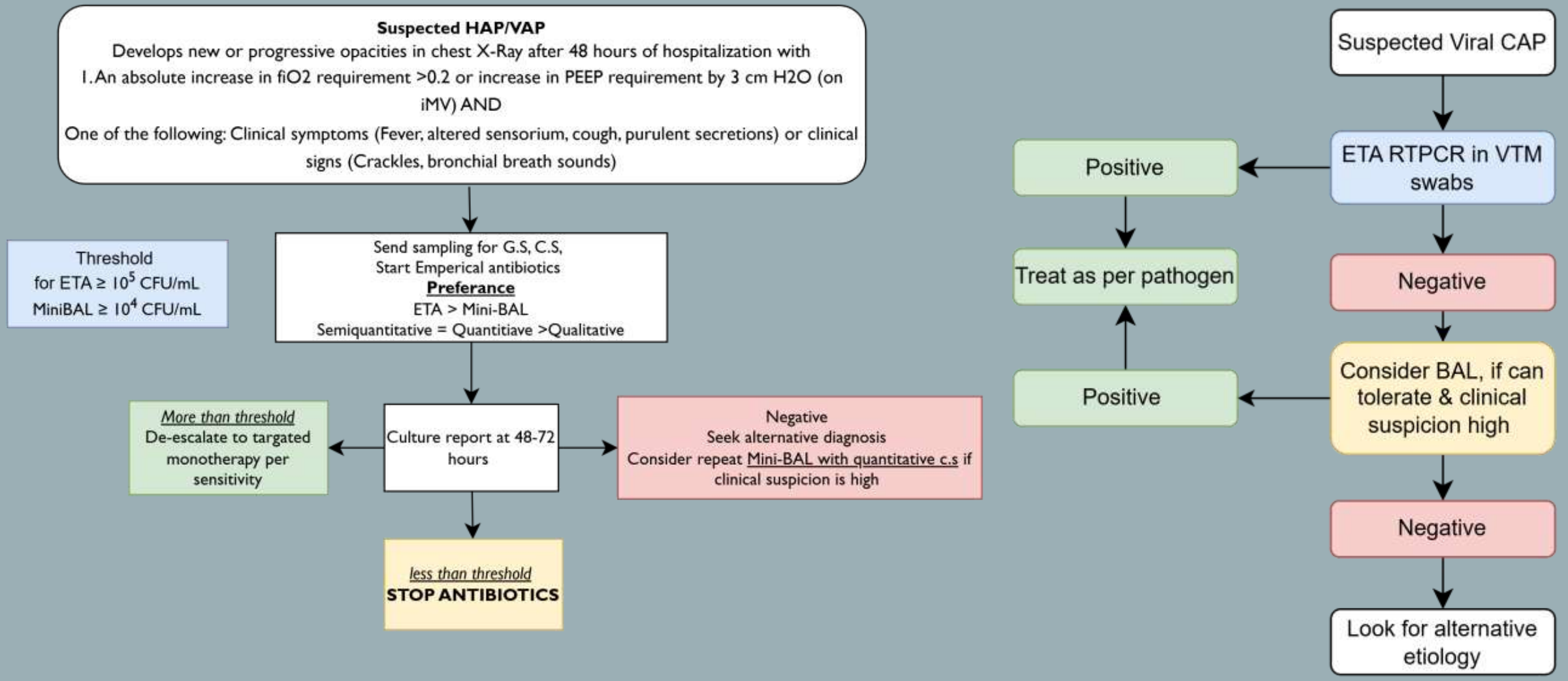
- BAL samples; being directed; is still heterogenous for CMV testing
- Proximal sampling like ETA might overestimate by detection of shedding or may not detect in less viral load cases

Not enough evidences to support use of ETA for CMV PCR

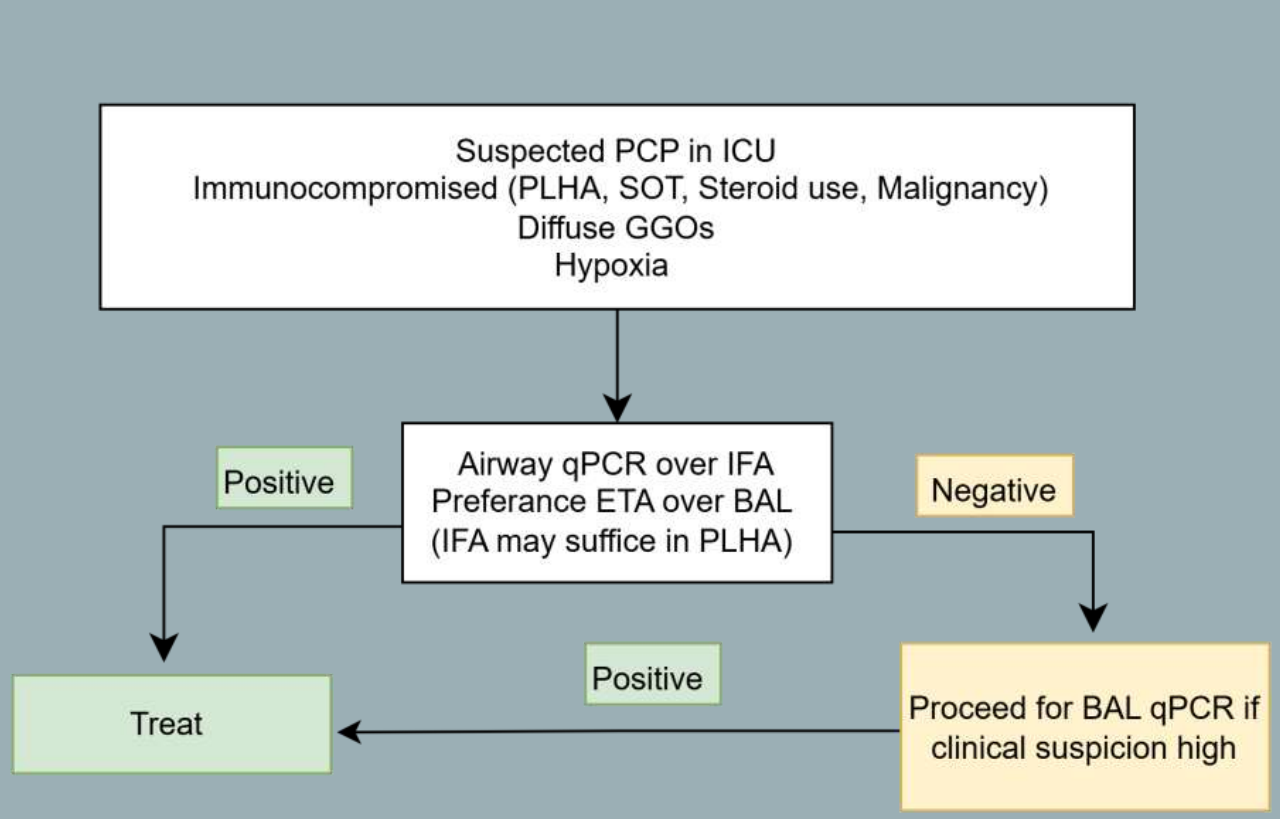
CMV pneumonitis

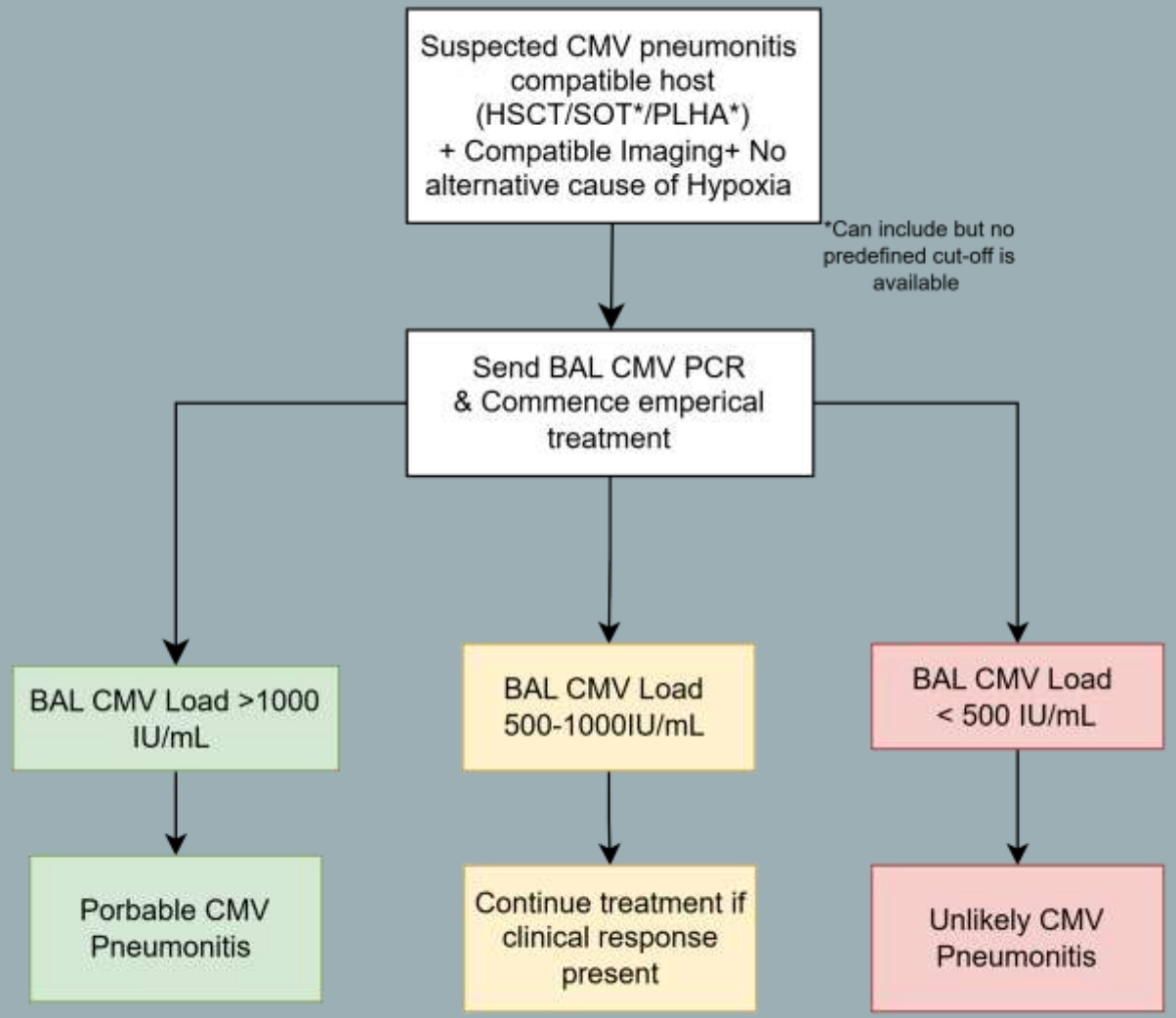


Takeaway Points



Takeaway Points





To conclude

- Non-bronchoscopic distal sampling (Mini-BAL) is promising: standardization is needed
- Proximal Sampling & Strict antibiotic stewardship in VAP
- Not rule out Viral CAP based on NPS
- Proximal sampling in fungal pneumonia needs further validation
- PCR is great equalizer in PCP detection
- High qualitative cut-off in BAL prevents treating benign shedding
-

Thank you

Happy to take questions.