Bacterial biofilms predominate in both acute and chronic human lung infections

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Background

A basic paradigm of human infection has long been that acute bacterial infection is caused by fast growing planktonic bacteria while chronic infections are caused by slow-growing biofilm bacteria. For lung infections, this paradigm has been thought to be supported by observations of how bacteria proliferate in well-established growth media in the laboratory—the gold standard of microbiology. However, this basic paradigm has not been quantitatively tested in samples from human infection sites. To address this gap in knowledge, we directly tested the existing paradigm that acute human infections are caused by planktonic cells and chronic infections are caused by biofilms. Therefor, sputum samples from patients with acute community-acquired pneumonia (CAP) without known chronic lung disease, acute pneumonia in chronic obstructive pulmonary disease (COPD) and chronic infections in cystic fibrosis (CF) were compared.

Aim

To investigate similarities and differences in the bacterial architecture and growth in sputum from patients with either acute (CAP and COPD) or chronic (CF) lung infections.

Conclusion

Biofilm growth is not reserved for chronic infections such as cystic fibrosis, but also predominates in CAP and COPD. The difference lies primarily in metabolic rates, not bacterial architecture. There is similar accumulation of the local inflammatory cells in acute and chronic pneumonia. Thus, we recommend to reconsider if the presence of aggregated bacteria can be used as a sole diagnostic marker for a chronic infection as well as the lack thereof may necessitate the infection to be acute.

Results

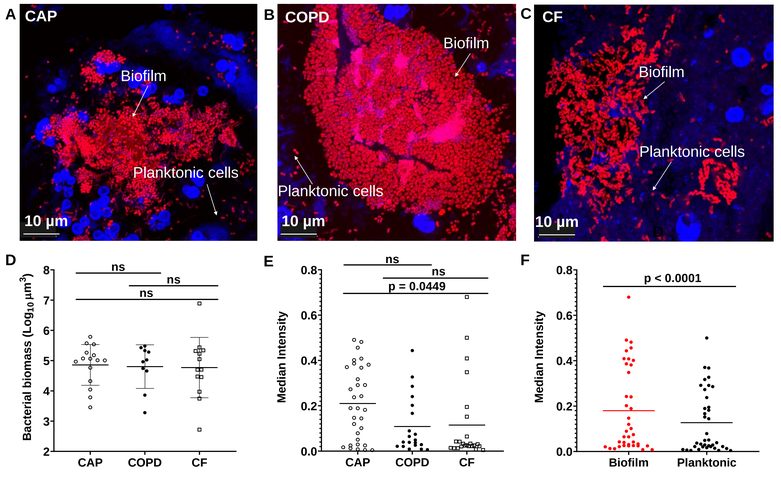

Figure 1: Biofilms and planktonic cells are observed across infection types. (A–C) Representative projections of confocal images of sputum samples of CAP with no detected pathogen (A), COPD with *Moraxella* sp (B) and CF with *Pseudomonas aeruginosa* (C). Specimens were stained with Tamra-5 (red) using PNA-FISH probes specific to bacterial 16S rRNA and DAPI m(blue). (D) Total biomass of bacteria by infection type. Bacterial biomass was calculated on each sample (n=43) by counting the voxels representing bacteria after image analysis pipeline.(E) Comparing the medians of the fluorescence intensity in each sample across infection type. (F) Bacterial objects on each sample were identified, classified as either planktonic cells (≤5 µm³) or biofilms (>5 µm³) and their per cent contribution to total biomass was calculated.

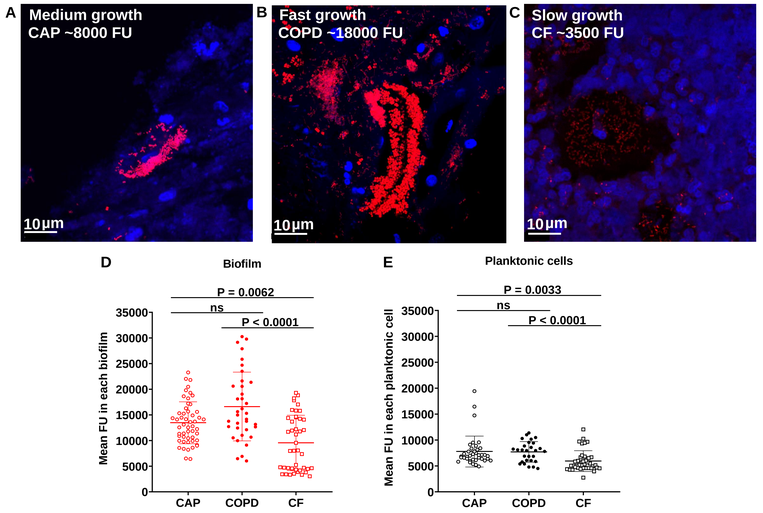

Figure 2: Growth estimates based on fluorescence in sputum samples. (A-C) Visualization of representative sputum samples from each patient group analysed with quantitative PNA-FISH based on fluorescence emitted from a universal bacteria specific PNA-FISH probe (16S rRNA) to demonstrate growing cells based on the increased numbers of ribosomal content. (D) Fluorescence intensity (FU) measured in each biofilm detected in images from sputum samples from CAP (n= 15), CF (n= 12) and COPD (n= 10). (E) Fluorescence intensity (FU) measured in all planktonic cells detected in images from sputum samples from CAP (n= 14), CF (n= 13) and COPD (n= 11).

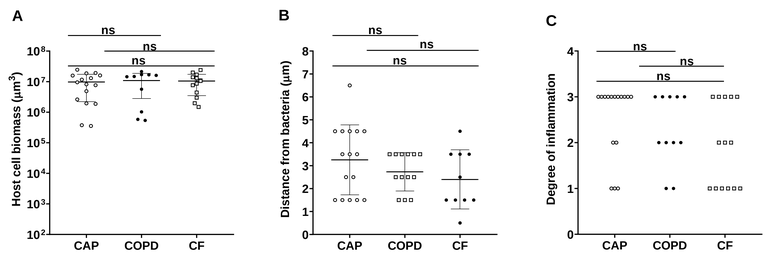

Figure 3. Similar inflammatory cell distribution and degree of inflammation. (A) Mean total biomass of inflammatory cells by infection type. Confocal images of sectioned sputum were stained with DAPI and biomass calculated by the total number of and blue fluorescent voxels. (B) Distance from bacteria at which the proportional occupancy of inflammatory cells is highest for each sample type. (C) Blinded histopathological evaluation of sputum samples of degree of inflammation from sputum samples: from CAP (n=16), COPD (n=11) and CF (n=14). Degree of inflammation: 0: no inflammation, 1: mild inflammation, 2: moderate inflammation and 3: severe inflammation.

Table 1. Characteristics of 43 patients with lower respiratory tract infection

	*CAP (n=16)	*COPD (n=13)	CF (n=14)
Median age in years (IQR)[a,b]	78 (69-84)	69 (61-81)	37 (25-48
Female, n (%)	6 (37.5)	5 (38.5)	4 (28.6)
Median body mass index (kg/m²) (IQR)	26 (24-29)	28.3 (23.7-29)	21.7 (20.8-26.7)
Median CRP (mg/L) on admission (IQR)[a,b]	43 (15-101)	20 (10-151.5)	3.5 (2-7)
Diabetes, n (%)	3 (18.8)	3 (23.1)	6 (42.9)
Median blood neutrophil count (10^8/L) on admission (IQR)	6 (5-10)	7.7 (5.2-10.8)	5.7 (3.9-9.5)
Duration of chronic infection (years)	NA	NA	22.5 (6-38)

Data are presented as % (counts), unless otherwise indicated. *Patients with CAP and COPD with enough material and without other lung diseases and antibiotic treatment before enrolment. [a]P <0.05 CAP vs CF and [b]P <0.05 CF vs COPD by one-way ANOVA test or Kruskal-Wallis test. ANOVA, analysis of variance; CAP, community-acquired pneumonia; CF, cystic fibrosis; COPD, chronic obstructive pulmonary disease; CRP, C reactive protein; NA, not available.

Materials and Methods

- 43 patients with suspected pneumonia were prospectively enrolled from two hospitals in Denmark: patients with CAP or COPD from Nordsjællands Hospital and patients with CF from the Copenhagen CF Centre at Rigshospitalet.
- Expectorated sputum samples were obtained from patients with CAP, COPD and chronic CF infections. Sputum samples (n=43) were fixed immediately after expectoration in phosphate-buffered saline that contained 4% paraformaldehyde and were embedded in paraffin. Sections of 4.0 µm and 30.0 µm were cut using a standard microtome and were fixed on glass slides.
- Sputum specimens were prepared and stained with a 16 A Tamra-5 (red)-conjugated peptide nucleic acid fluorescent in situ hybridisation (PNA-FISH) probe specific for bacterial 16S rRNA and 4′,6-diamidino-2- phenylindole (DAPI) was used to stain all bacteria present in the sputum. Specimen slides were scanned using a confocal laser scanning microscope (CLSM) (Axio Imager.Z2, LSM 880, Zeiss, Germany).
- Raw images were processed using Imaris V.8.2 (Bitplane). Objects ranging in size from 0.5 µm³ to 5.0 µm³ were classified as planktonic cells. Objects larger than 5.0 µm³ were classified as aggregates, and objects smaller than 0.5 µm³ were excluded from analysis.