Sputum color as a marker of acute bacterial exacerbations of chronic obstructive pulmonary disease

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Summary We analyzed 795 sputa from 315 patients (233 males, mean age 69.3 ± 8.8 years, mean number of exacerbations 2.52/patient) with acute exacerbations of moderate-to-severe chronic obstructive pulmonary disease (COPD) (mean steady-state FEV1, 42.5 ± 7.8% of predicted). 581/795 sputa were considered adequate. Sputum was analyzed by a quali-quantitative colorimetric scale allowing both color distinction and color degree of intensity. Quantitative culture was then performed (threshold: > 10⁶ CFU/mL). Samples were distinguished in mucoid (145) and purulent (436) sputa. Absence of bacterial growth was observed in 22% and 5% of mucoid and purulent sputa, respectively. Among mucoid sputa, Gram positive bacterial growth occurred more commonly compared to Gram negative and Pseudomonas aeruginosa/Enterobacteriaceae (56%, 24%, 20%, respectively). In purulent sputa, Gram positives were found in 38% of cases, Gram negatives in 38%, and P. aeruginosa/Enterobacteriaceae in 24%. We evaluated whether functional impairment (FEV1) orientates as to the infectious etiology of exacerbations. Significant differences were observed in the distribution of pathogens. Gram negative and P. aeruginosa/Enterobacteriaceae were isolated more frequently in the sputum when FEV1 was < 35%. Our study indicates that purulent sputum is strongly associated with bacterial growth in COPD exacerbations. Deepening sputum color (from yellowish to brownish) was associated with increased yield of Gram negative and P. aeruginosa/Enterobacteriaceae.

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Introduction

Chronic obstructive pulmonary disease (COPD) is a common respiratory disease associated with substantial health burden worldwide. It is estimated that COPD affects at least 52 million worldwide, and accounted for 2.74 million deaths in the year 2000.1 The natural history of the disease is one of progressive decline punctuated with varying frequency by exacerbations of symptoms.2 Available data suggest that patients have such exacerbations at a median rate of 2–3 episodes per year.3

There is no consistent definition of an acute exacerbation of chronic bronchitis (AECB), but these events generally include some, or all, features such as increased dyspnea, wheeze, cough, sputum volume, sputum purulence, chest tightness, hypoxemia and hypercapnia. The best known clinical definition was that proposed by Anthonisen et al.,4 who identified increased sputum volume, sputum purulence and dyspnea as cardinal manifestations of AECB.

The causes triggering an acute exacerbation are generally multifactorial and commonly include a combination of smoking habits, environmental irritants and infection.5 It is thought that up to 70% of infectious cases are caused by bacteria, the remainder being attributable to “atypical” pathogens such as viruses, Mycoplasma pneumoniae, and Chlamydia pneumoniae.6 Therefore, the use of antibiotic therapy in the management of exacerbations seems a rational choice in a certain number of cases, although the matter is still debated. A meta-analysis of randomized, placebo-controlled trials of patients on antibiotic treatment in AECB demonstrated a small but statistically significant improvement attributable to antimicrobial therapy.7

Various attempts have been made to correlate exacerbation characteristics with putative bacterial pathogens. Miravitlles et al. have shown that microorganisms causing acute exacerbations of COPD are distributed unevenly among patients with different degrees of disease severity, with patients more severely affected showing a greater incidence of Pseudomonas aeruginosa and Haemophilus influenzae.8 Sethi et al. associated increased neutrophil airway inflammation, as assessed by IL8, TNF-α, and neutrophil elastase, with isolation of bacteria in sputum of chronic bronchitis patients.9 Recently, Stockley et al. devised a sputum color chart in order to distinguish between purulent and mucoid COPD exacerbations.10 The underlying principle was that the presence of bacteria causing infection in the airways would be associated with neutrophil recruitment, thus correlating with the degree of yellow-green coloration of the sputum due to neutrophil myeloperoxidase accumulation. The authors found that the presence of green (purulent) sputum was specific for a high bacterial load in the airways, indicating a subset of patients that would most likely benefit from antibiotic therapy.

Aim of the present study was to determine whether sputum color can provide an orientation as to the infectious etiology of COPD exacerbations. We also investigated the possible associations between the degree of functional impairment, as measured by FEV1, and presence of bacterial pathogens in the airways.

Materials and methods

Between 1997 and 2002 we considered patients with COPD exacerbations referring to our institutions. All had a clinical history of chronic bronchitis (daily sputum production for at least 3 months of 2 consecutive years), patients with a past history of bronchiectasis were excluded. We analyzed 795 sputa from 315 patients (233 males, mean age 69.3±8.8 years) with acute exacerbations of moderate-to-severe COPD (mean steady-state FEV1 42.5±7.8% of predicted). Patients were placed in either the FEV1 50–69.9% category (257 patients), or the FEV1 <50% (58 patients). Acute exacerbation was defined by increased dyspnea, cough, sputum volume, and sputum purulence as reported by the patients. Patients were excluded if they had received antibiotic treatment during the previous 4 weeks. On the day of consultation, demographic details, steady-state spirometry and post-bronchodilator FEV1 values (obtained within the previous 6 months) were recorded from the clinical chart. In 477/795 episodes, patients were hospitalized. Written informed consent was obtained from all patients. The study was approved by the University of Milan Ethical Review Board.

A fresh sputum sample was obtained in a sterile container, as free from saliva as possible. Gram stain was performed, and sputum validity was assessed by means of the Bartlett criteria. Quantitative sputum culture was then performed (positivity threshold: >104 CFU/mL).

In addition, sputum was analyzed by a qualitative colorimetric scale (patent no. 00218892/1994) characterized not only by color distinction but also by degree of intensity. Sputum samples were compared with the colors of a multicolor “ink catalogue” (gamma “Pantone”). All colors in sputum samples were in the catalogue.
Only for the definition of “white” in mucous sputum was it necessary to resort to a blend of colors. We identified about 50 different colors, including shades or tones of individual colors. For practical reasons we selected 10 reference colors together with a “physiological” white. In addition to being the most commonly occurring colors, the 10 selected specimens were used as reference “average” colors for other shades and tones. Sputum samples were collected in a funnel. A colorimetric scale was let slide under the funnel, thereby allowing the approximate color identification. Each color is coded with a number, which can later be compared with other sputum samples (Fig. 1). Samples with white or gray preponderance (numbers 0 and 1, respectively) were considered as mucoid, whereas shades of yellow, green, and brown were considered as purulent (numbers 2–9). In a subgroup of the first 100 patients enrolled, we compared sputum color assessment provided by the patient and one of the investigators (PLD) using, as gold standard, the colorimetric scale.

Statistical analysis

Statistical differences between groups were determined by the Student t-test for unpaired data.

Results

Sputum characteristics and bacterial growth

Sputum samples were considered adequate in 581/795 exacerbations (73%) according to the Bartlett criteria. Samples were distinguished in mucoid (145) and purulent (436) sputa.

Absence of bacterial growth was observed in 32/145 (22%) and 22/436 (5%) of mucoid and purulent sputa, respectively (P < 0.001). Among mucoid sputum samples (color grading 0–1), Gram positive bacterial growth occurred more commonly compared to Gram negative and P. aeruginosa/Enterobacteriaceae (62.9%, Streptococcus pneumoniae 86%, Streptococcus spp. 11%, Staphylococcus aureus 3%), 18.8% (H. influenzae 30% M. catarrhalis 70%), and 18.2%, respectively. In purulent sputa, Gram positive bacteria were found in 44.6% of cases in yellowish sputa (color grading 2–4) (S. pneumoniae 85%, S. aureus 10%, Streptococcus spp 5%), in 22.6% in greenish sputa (color grading 5–7) (S. pneumoniae 92% S. aureus 4%, Streptococcus spp. 4%), and in 18.9% in brownish sputa (color grading 8–9) (S. pneumoniae 42% S. aureus 58%). Gram negatives were isolated in 49.9% of cases in yellowish sputa (H. influenzae 70%, H. parainfluenzae 10%, M. catarrhalis 20%), in 39% in greenish sputa (H. influenzae 53%, H. parainfluenzae 15%, M. catarrhalis 32%), and in 37.8% in brownish sputa (H. influenzae 64%, H. parainfluenzae 16%, M. catarrhalis 20%).

P. aeruginosa/Enterobacteriaceae were found in 5.4% of cases in yellowish sputa, in 38.3% in greenish sputa, and in 43.2% in brownish sputa. Considering sputum purulence and isolated bacteria, Gram positive bacteria isolation was significantly associated with mucoid sputum (P < 0.001). Conversely, Gram negative and particularly Pseudomonas/Enterobacteriaceae isolation was associated with purulent sputum (P < 0.001). Moreover, Pseudomonas/Enterobacteriaceae isolation was significantly associated with higher color grading (> 5; from green to brown) (P < 0.001). Table 1 shows the correlation between sputum color and bacterial growth.

Lung function

Significant differences were observed in the distribution of pathogens on the basis of the degree of functional impairment. Gram negative and P. aeruginosa/Enterobacteriaceae were isolated more frequently (P < 0.001) in the sputum of individuals with FEV1 < 35% (Fig. 2).

Color evaluation

The definition of sputum color by patients and investigators was Concordant in 68% of the cases, without the aid of the color stick. When this first judgment was compared to evaluation with the color stick, patient and investigator definitions
were confirmed only in 41% and 45% of the cases, respectively. Discrimination of whitish, grayish, yellowish, and brownish color shades was complex, both for patients and investigators. In practice these shades were employed interchangeably. In addition, significant overestimations were found for the definition of greenish (patient-color stick = 10:1; investigator-color stick 11:1) and brownish (patient-color stick 8:1; investigator-color stick 3:1) (Table 2).

**Discussion**

In recent years, a greater insight has been achieved in our knowledge on the inflammatory processes involved in exacerbations, and on the relevance of bacterial colonization in disease progression. A major finding in the airway secretions of patients with AECB is an increase in neutrophil accumulation, high granulocyte/macrophage colony stimulating factor, and increased markers of neutrophil activation such as myeloperoxidase. There appears to be a hierarchical contribution of chemoattractants, with LTB4 being the first to change at low levels of neutrophil influx (mild exacerbations), and IL-8 becoming involved as neutrophil influx and activation becomes more intense in more severe exacerbations. In a subset of COPD patients with mild exacerbations prominent eosinophilia was found both in the airway wall and the airway lumen. The postulated mechanism for eosinophil recruitment is an increase in TNF-α production which activates epithelial cells and sub-epithelial lymphocytes to produce the chemokine RANTES.

Sputum purulence is one of the potential markers of bacterial infection during exacerbations. Recent studies indicate that purulence is associated with significant bacterial growth and high microbial load. Conversely, the bacterial nature of “mucoid” exacerbation has been questioned. In fact, Stockley et al. reported that mucoid sputum yielded positive bacterial culture in 38% of 34 exacerbations whereas purulent sputum culture resulted positive in 84% of 87 exacerbations. Moreover, in mucoid exacerbations the bacterial load was low and only two patients deteriorated without antibiotics. When patients were restudied in a stable clinical state the sputum characteristics were unchanged.

Our study indicates that purulent sputum obtained during exacerbation is almost invariably associated with bacterial growth in patients with moderate-severe COPD. Furthermore, deepening sputum color (from yellowish to brownish) is associated with increased yield of Gram negative and *P. aeruginosa/Enterobacteriaceae*. These bacteria are also more common in patients with most severe functional impairment.

Color definition according to a colorimetric stick provides a better approximation than patient or investigator color descriptions such as “yellowish”, “whitish”, or “greenish”, etc. We found that patients are often very imprecise in identifying colors; what is described as yellow is often, in fact, green, and white is often actually gray. We were able to overcome this difficulty by furnishing an “official” reference color by sliding a colorimetric stick along the tube containing the specimen being examined and reading the number corresponding to that color.

Compared to data in the paper by Stockley et al., we recorded a very high culture positivity results both in mucoid and purulent sputa. Possible explanations may be that the patient populations in the two studies were not fully comparable since participants in our study showed a higher degree of functional impairment, and that a significant proportion required hospitalization for their exacerbation. Moreover, out of the total number of sputa, 92.6% were cultured in the study by Stockley et al. compared to 73% in our study. This might be due to more selective screening criteria in our population and may be associated with a higher probability of observing positive bacterial growth.
Our data indicate that patient and investigator judgment of color shade definition is unsatisfactory, leading to underestimation of darker shades of sputum coloration compared to the reference color stick. This suggests that use of a colorimetric stick may be important in order to correctly identify patients that show a higher probability of harboring Gram negative bacteria in their airways.

In summary, our study indicates that purulent sputum is almost invariably associated with bacterial growth in patients with COPD exacerbations. Furthermore, deepening sputum color (from yellowish to brownish) is associated with increased yield of Gram negative and *P. aeruginosa/Enterobacteriaceae*. These bacteria are more common in patients with most severe functional impairment.

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**References**


