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# Microsatellite DNA Instability and Chronic Obstructive Pulmonary Disease Exacerbations

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**Abstract**

**Background:** Increased frequency of Microsatellite DNA Instability(MSI) has been detected in sputum of COPD patients. Our aim was to investigate the relationship between MSI in sputum cells and exacerbation frequency which is an important parameter in the clinical course of the disease.

**Methods:** Induced sputum samples and peripheral blood obtained from 36 patients with COPD at stable state were analyzed. The control group consisted of 30 non-smokers healthy subjects. DNA was extracted and analyzed for MSI using the following Microsatellite markers: RH70958, D5S207, D6S2223, D6S344, D6S263, G29802, D13S71, D14S588, D14S292, D17S250. Following MSI analysis, exacerbations were recorded for three years in total.

**Results:** No MSI was detected in healthy non-smokers. Eighteen(18) out of thirty six(36) patients (50%) exhibited MSI in their sputum cells ($p<0.0001$). Patients exhibited MSI showed significantly increased frequency of exacerbations compared to patients that did not ($p=0.003$). In addition, a significant increased frequency of purulent ($p=0.002$) and of severe type exacerbations ($p=0.001$) was found in patients exhibiting MSI. Patients positive for marker G29802 or D13S71 or D14S588 presented increased exacerbation frequency ($p<0.01$).

**Conclusion:** The significant association between MSI and COPD exacerbations indicates that somatic mutations could be involved in the pathogenesis and natural history of the disease.

**Word count**: 200

**Abbreviations**

COPD = Chronic Obstructive Pulmonary Disease

MSI = Microsatellite DNA instability

MMR = DNA mismatch repair system

PCR = polymerase chain reaction

## Introduction

The course of Chronic Obstructive Pulmonary Disease (COPD) is characterized by exacerbations that induce inflammation and aggravate oxidative stress in the lungs leading to further physiologic deterioration[1][2]. Notably, some COPD patients present the frequent exacerbation phenotype of the disease. The severity of the disease and the number of exacerbations in previous years have been associated with an increased exacerbation frequency[3][4]. However, it is not known whether exacerbation frequency is associated with genetic alterations or whether there is a genetic susceptibility for frequent exacerbations, despite the fact that previous studies led to the identification of several genes probably involved in COPD pathogenesis[5][6].

In our previous investigations, we reported that COPD patients present a high frequency of genetic alterations at the microsatellite DNA level, which are detected in their sputum cells[7][8]. Microsatellite DNA consists of very short tandem nucleotide repeats that are found scattered throughout the human genomes of eukaryotes[9][10][11][12]. Insertion or deletion of these DNA sequences (Microsatellite DNA instability or MSI) has been correlated with a high somatic mutational rate and is associated with a defective DNA mismatch repair (MMR) system[13][14]. Thus, MSI detection in sputum cells of COPD patients could be a useful marker, indicating destabilization of the genome at various loci.

In this respect, we investigated whether there is a relationship between somatic genetic alterations and exacerbation frequency in COPD and

therefore, whether patients who exhibit Microsatellite DNA instability in their sputum cells present an increased exacerbation frequency compared to patients exhibiting no MSI.

## Methods

### Subjects and protocol

A total of 66 subjects were studied. Baseline characteristics of 36 COPD patients are shown in Table 1. The GOLD Consensus Statement was used for the diagnosis of COPD[15]. The control group consisted of 30 healthy non-smoking subjects [mean(SD) age 56±17 years], with $FEV_1$(%pred): 92±4, FVC(%pred): 88±4 and $FEV_1$/FVC: 83±8. Patients were recruited by consecutive sampling from a cohort of a longitudinal study of lung function decline in COPD[3]. Patients with Asthma, upper or lower respiratory tract infection within last 4 weeks before the study, history of lung (or another) cancer were excluded from this study.

This investigation was a three-year prospective study, incorporating a run-in period of 4 weeks, sputum induction and clinical assessment at baseline[15][16] and outpatient clinic visits, scheduled every 3 months. At baseline patients underwent sputum induction at steady condition, sputum was cultured for bacteria and differential sputum cell count was estimated and sputum and blood were examined for MSI. All 36 patients were then provided with diary cards to record changes in symptoms [dyspnea, sputum production, purulence (major symptoms) and wheeze, cough, nasal discharge, sore throat, fever (minor symptoms)]. A patient directed diary card and clinic records were used to identify and assign

exacerbations. A protocol based on GOLD was used for individual exacerbation treatment[15]. The study was approved by Medical Research Ethics Committee of the Hospital and patients gave their informed consent.

**Exacerbations**

The definition of an exacerbation was based on previously described criteria[4][17] requiring either, increase of at least two major respiratory symptoms (dyspnea, sputum amount, and sputum purulence) or, increase of one major symptom in addition to at least one minor symptom (wheeze, cough, fever, nasal discharge, sore throat), for at least two consecutive days. The first day with increased symptoms was taken as the onset of the exacerbation. Following an exacerbation, patients were required to have a two-week period (recovery period) with the same or less symptoms as those present before the start of an exacerbation before another exacerbation was studied.

**Monitoring of exacerbation**

**Diary card.** The development of the diary card was based in previously used diary card[3]. All patients were instructed to record, at the end of each day, any increase in symptoms in respect of the last 24 hours. Changes in symptoms were registered using a binary-coded system. Patients were seen in scheduled visits as outpatients every three months and diary cards were collected. Exacerbations identified from diary cards were termed “diary card recorded exacerbations”.

**Medical records**. Exacerbations when no diary card symptoms were recorded were identified either by questioning the patients about

symptoms at the clinic visits or on admission to hospital or by reviewing of hospital medical records. Exacerbations identified by this way were termed as “medical-records identified exacerbations”

**Exacerbation rate**

The total number of exacerbations was obtained by combining the number of “diary-card recorded exacerbations” plus the number of “medical-records identified exacerbations”. The total annual rate for each patient was calculated by dividing the total number of exacerbations by the number of days participated in the study and then multiplying by 365.

**Sputum induction**

Sputum was induced via inhalation of a hypertonic saline solution aerosol, generated by an ultrasonic nebulizer (Ultraneb 2000; DeVilbiss; Somerset, PA). A previously described procedure was followed for sputum induction, processing, total and differential cell counting[8].

**DNA extraction from sputum and blood cells**

The presence of MSI in sputum cells in comparison with DNA obtained from peripheral White blood cells from the same individual was investigated. DNA extraction was carried (Qiagen Extraction Kits, QIAmp DNA Blood Maxi and Mini Kits, QIAGEN Inc) and DNA samples were stored at –20°C.

**Microsatellite markers and Microsatellite DNA instability analysis**

Ten polymorphic Microsatellite markers were used to assess MSI (RH70958, D5S207, D6S2223, D6S344, D6S263, G29802, D13S71,

D14S588, D14S292, D17S250) (see Table 2). The above markers were chosen as they are located closely to genes possibly related to COPD pathogenesis[6][7][8]. The sequences of the Microsatellite markers used were provided through the NCBI Database (www.ncbi.nlm.nih.gov). The polymerase chain reaction (PCR) technique was used to amplify DNA sequences. PCR amplifications were carried out in 50 μl final volume reaction mixtures in a PTC-100 thermal cycler (M.J.Research Inc., Watertown, MA, USA), using the Qiagen *Taq* PCR Core Kit (QIAGEN Inc). Forward primers were labelled with the Licor IR800 fluorochrome. The following thermal cycling protocol was applied: 3 min at 94°C, 30cycles at 94°C for 30 sec, 55°C for 30 sec, 72°C for 30 sec, 72$^{o}$C for 5 min and terminated at 40°C. The PCR products were analysed and visualised by electrophoresis in 8% Long Ranger polyacrylamide (BMA, Rockland, Me, USA) 7 M urea sequencing gels in a Licor 4200 DNA sequencer (Lincoln, NE, USA) and alleles were sized with GeneProfiler v3.54 software (SCANALYTICS, USA). MSI was identified by comparing the electrophoretic patterns of the Microsatellite markers of DNA of sputum versus peripheral blood demonstrating a shift of one or both of the alleles, thus, generating novel alleles as indicated by an addition or deletion of one or more repeat units. Figure 1 shows representative sample of Microsatellite DNA stability, as well as Microsatellite DNA instability (MSI), in the Microsatellite marker D6S344. Two scientists who were not aware of the clinical characteristics of the subjects performed independent readings. All MSI-

positive samples were tested twice using fresh DNA, showing 100% reproducibility.

**Statistical analysis**

Normality of the numerical parameters was tested using the Kolmogorov-Smirnov test. Student's t-test for normally and the Mann-Whitney test for non-normally distributed data were used to estimate significant difference among the groups. Chi-squared test was used for comparison of percentages. Data analysis was carried out by using the SPSS statistical software package (SPSS Inc., Chicago, IL, USA; version 11.0). A p value $<0.05$ was considered statistically significant.

## Results

**Exacerbations**

Table 3 shows overall number of exacerbation in the 3-years period. The participants were followed for median (min-max) 1095 (760-1095) days and recorded their symptoms on the cards for median (min -max) 745 (490-1095) days. The types of exacerbations are presented in Table 3. More than 50% of the exacerbations were characterized as purulent. Forty eight (15.4%) exacerbations required hospitalization which occurred at the same hospital in 46 cases. The mean (95% CI) annual rate of admissions due to exacerbations was 0.44 (0.2-0.68). Patients (n=10) with bacterial colonization at baseline had significantly higher median(IQR) 3-year annual exacerbation rate than patients without evidence of colonization (n=26) [4.4(2.3-5.3) vs 2.6(1.3-3.3), p=0.01].

**Microsatellite DNA instability (MSI)**

Eighteen out of thirty six COPD patients (50%) exhibited MSI in their sputum cells [MSI(+)ve subjects]. All healthy subjects showed no MSI in any of the 10 Microsatellite markers tested.

Subjects' characteristics were then compared according to the presence of MSI or not (Table 4). No statistically significantly differences were found in the anthopometric, clinical data including chronic bronchial colonization between the two groups (Table 4)**.** In addition, the median(IQR) number of days between sputum induction and last exacerbation was not significantly different between MSI(+)ve and MSI(-)ve subjects [60(45-137) vs 60(45-120), p=0.4 respectively]. The degree of airway inflammation in stable state was not different between the two groups in terms of inflammatory cells profile (Figure 2). No statistically significant relationship was found between $FEV_1$(% pred) and MSI frequency.

Table 2 also shows that MSI was detected in more than one marker in the same individual (33 MSI in 18 patients). The most frequently affected markers were D13S71 and G29802 (positive in 8 and 7 subjects respectively).

**The relationship between MSI and exacerbations**

Figure 3 shows that patients who exhibited MSI, experienced a significantly higher number of exacerbations compared to patients who did not present MSI (p=0.003). When exacerbations were analysed according to sputum quality/severity a significant increased number of purulent (p=0.002) and type I (p=0.001) exacerbations was found in patients who exhibited MSI compared to patients who did not.

Patients who were positive for MSI marker G29802 or D13S71 or D6S344, had relatively higher overall exacerbation rates compared to patients who did not exhibit MSI (Figure 4). When exacerbations were analyzed by severity, Type I exacerbations rates were found to be significantly higher in patients positive either to marker G29802 ($p=0.004$), or to marker D13S71 ($p=0.007$), compared to patients who did not exhibit MSI.

**Discussion**

The purpose of this prospective study was to investigate the relationship between somatic genetic alterations and exacerbation frequency in COPD patients. In agreement with our previous investigations[7][8][17], a significant proportion (50%) of COPD patients in this study exhibited MSI in markers which are located closely to genes encoding for proteins possibly related to COPD pathogenesis. Moreover, 6 out of 18 (33%) subjects showed MSI in more than one marker (Table 2). The novel finding in this study is the significant association between MSI incidence and frequency of exacerbations. COPD patients positive to MSI were found to have a significantly higher overall exacerbation frequency than patients with no Microsatellite instability; this was particularly true for more complex (Type I) and for purulent exacerbations. In addition, analyzing MSI for each marker separately, we found that patients who exhibited MSI in the chromosomal region related to genes encoding either TNF or serpines [Protease inhibor 6 (PI6), and Protease inhibitor 9

(PI9)] or Perforin, experienced significantly higher number of overall exacerbations compared to patients who did not exhibit MSI (Figure 4).

Despite that sputum is a relatively heterogeneous sample to consider and the fact that it is not yet known which cell subpopulation is responsible for MSI, our previous studies provided evidence suggesting that the detection of MSI in sputum may be of importance in COPD. First, COPD patients presented sputum MSI in different chromosomal regions compared to sputum from asthmatics[8]. Second, MSI was found in sputum but not in nasal cytologic samples of COPD patients despite the fact that inflammation coexists in the nasal mucosa of these patients[17]. The latter suggests that MSI in the markers studied is a specific finding for the target organ of COPD, i.e. the lungs. Thus, MSI detection in COPD raises the questions when and how acquired somatic mutations at the Microsatellite level occurring during the course of COPD, play a role in the pathogenesis or in the natural course of the disease. However, the precise role of MSI detection in COPD has not yet been established.

MSI is seen in chronically inflamed tissues[18]. It has been suggested that oxidative stress can modify the inflammatory response and it can inactivate the human mismatch DNA repair system in a dose-dependent manner[19]. Thus, MSI can clearly be subject to a secondary change related to airway inflammation and increased oxidative stress which characterize COPD. In this respect, we assessed the relationship between MSI and COPD exacerbations which augment further airway inflammation and oxidative stress compared to stable state[20][21][22].

Remarkably, we found that patients who exhibited MSI in their sputum experienced a significantly higher number of exacerbations (Figure 3). This was true particularly for exacerbations categorized clinically as more complex or purulent. On this basis, it is tempting to hypothesize that repetitive stimulation by frequent exacerbations aggravates inflammation and oxidative stress in the airways and leads to an altered DNA repair process and therefore to an increased detection of genetic alterations at the Microsatellite level.

One might argue that MSI may represent insult from other factors which are not related to exacerbations per se. We certainly can not exclude that other environmental or clinical factors may result in MSI. However, the population studied represented mainly COPD patients followed at a specialist hospital clinic and it was in many terms homogenous (white, greeks, inhabitants of an island). Thus, we assume that all of the subjects were affected by the same environmental factors. In addition, comparing subgroups of COPD patients that were respectively positive and negative for MSI (Table 4), no statistically significant difference was found in relation to age, duration of illness, baseline treatment including steroids, bacterial colonization, smoking status and spirometric indices. Therefore, despite that the association between MSI and exacerbation frequency-complexity does not prove a causal relationship, our results deserve consideration. Our findings suggest that there is a potential relationship between an increased frequency of exacerbations and somatic acquired mutations at the Microsatellite DNA level which might be considered in the investigation of the pathogenesis of the disease. A

longitudinal investigation of MSI, using markers especially related to oxidative stress (ie. Heme-oxygenase), before and after a long follow up for exacerbations monitoring will potentially provide further information for the relationship between MSI, oxidative stress and exacerbations.

An alternative explanation for the relationship between MSI and exacerbations could be that MSI indicates predisposition to frequent exacerbations. Microsatellite instabilities may represent dysfunctional changes in genes directly causing increased exacerbations (i.e. within causal genes), or they may be in linkage disequilibrium with nearby causal genes. From this perspective, host response to environmental insult (viruses and bacteria) may be impaired and some COPD patients may experience frequent exacerbations, at least in part, due to molecular deregulations of host defenses[23][24]. Notably, clusters of smoking-induced somatic mutations has been reported previously in genes related to nuclear factor kB and activator-protein-1 activation which coordinate the expression of a plethora of inflammatory mediators[25][26]. This alternative hypothesis is also supported by findings of previous studies reporting that patients who presented frequent exacerbations were those who had increased exacerbation rates in previous years[4]. In addition, although advanced disease may be a significant parameter of increased exacerbation rate, not all patients with severe COPD are prone to frequent exacerbations[27]. Thus, genetic predisposition for increased exacerbation frequency is not unlike.

Furthermore, we might speculate that since MSI indicates defective DNA mismatch repair system, its presence might be also associated with a mechanism that inhibits the resolution of the inflammation post exacerbation. Thus, these mutations might contribute to the pathogenesis of airway inflammation that is observed in stable COPD and consequently to an increased exacerbation frequency. Notably, frequent exacerbators have increased airway inflammation when stable[22]. A longitudinal study is under way to investigate whether MSI represent dysfunctional changes in genes or, it is a marker of epithelial or other cell turnover/mutation.

It should be underlined here that our findings do not suggest that MSI is the only responsible mechanism for frequent exacerbation or that the role of other parameters that affect exacerbation frequency, such as the bacterial airway load, is negligible[28]. Indeed, an increased exacerbation frequency was associated with the presence of bacterial colonization at baseline stable state in this study. In contrast, the presence of bacterial colonization was not associated with MSI. On this basis, MSI may be a biomarker of a causal mechanism -not directly related to bacterial colonization- that leads to increased frequency of exacerbations.

We certainly acknowledge that quantitative sputum cultures were not performed and viral colonization which may be also important in influencing the rate of exacerbation was not assessed in this study. This limitation should be considered in the interpretation of our results. In

addition, the hospitalization rate in this study may be higher than rates previously reported. This is probably due to the structure of the health care system in Greece where public primary health care is still developing. Hence, many patients are admitted to hospitals even for short hospitalizations due to the luck of a high standard public service in the community. However, this fact may have not significantly affected our results because detection of exacerbations and evaluation of their severity was based on daily monitoring of symptoms by using diary cards.

In conclusion, the instability of the Microsatellite sequences that we have investigated indicates a destabilization of the genome in COPD patients. Oxidative stress may damage the DNA of lung cells, probably at the Microsatellite level, leading to acquired somatic mutations. These mutations, expressed as Microsatellite instability, permanently alter DNA auto-repair ability[29]. This phenomenon was demonstrated especially in COPD patients with an increased number of exacerbations, assessed during a three-year period. We may speculate that the relatively high exacerbation rate that was found in COPD patients with genetic instability could provide a link between alteration in DNA MMR activity and oxidative damage due to frequent COPD exacerbations and vice a versa. Therefore, it would be of interest to consider not only the inherited alterations in the DNA but the somatic acquired mutations at the Microsatellite DNA level, as this may provide novel information in the pathobiology of COPD.

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## Figure legends

**Figure 1:** Representative sample of Microsatellite DNA Instability (MSI), in the marker D6S344, analysed with LI-COR Saga GT Microsatellite Analysis Software. The expected PCR product size for the locus D6S344 is between 139-159bp (NCBI UniSTS:36924). Hereby, the software Saga is given the range between which the product is expected (139-159bp); starting from 139 the program puts colored lines every 2 bp, providing the exact size of each allele. The figure shows a microsatellite instability, where each allele is characterized according to its size. The blood DNA sample shows one allele in 143bp painted red (together which its corresponding peak) and the other in 151bp painted pink. In contrast, the sputum DNA sample is translocated showing clearly the instability: the first allele is in 141bp (colored dark yellow) and the second in 147bp (bright yellow). The greater the peaks are the most amplified the sequence is. Lower peaks (blue line) only show by-products of the reaction. *(BL: DNA specimen obtained from peripheral blood; SP: DNA specimen obtained from sputum; bp: base pair).*

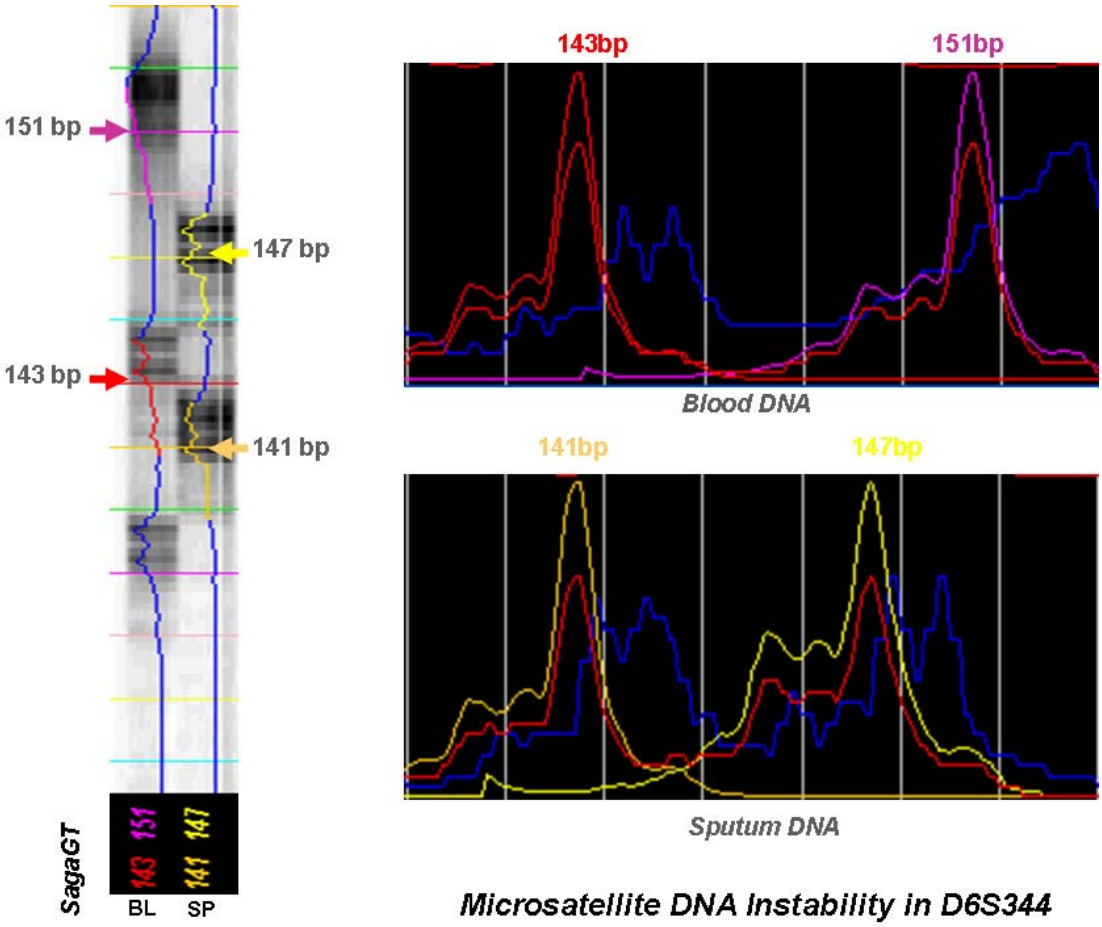



**Figure 2.** Sputum differential cell counts in COPD patients with positive and negative Microsatellite DNA Instability (MSI) markers. Data are presented as median (interquartile range)

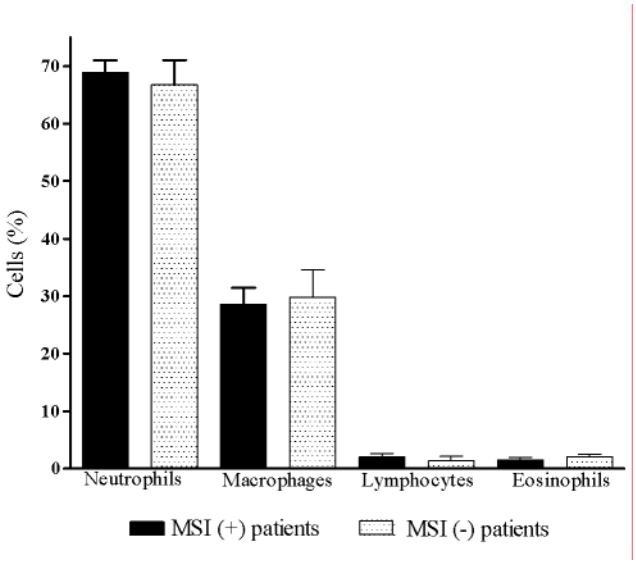


**Figure 3.** Individual annual exacerbation rates of COPD patients with positive and negative Microsatellite DNA Instability (MSI) markers, overall and according to sputum purulence and severity (type I, II, III exacerbations). Horizontal lines represent median values.

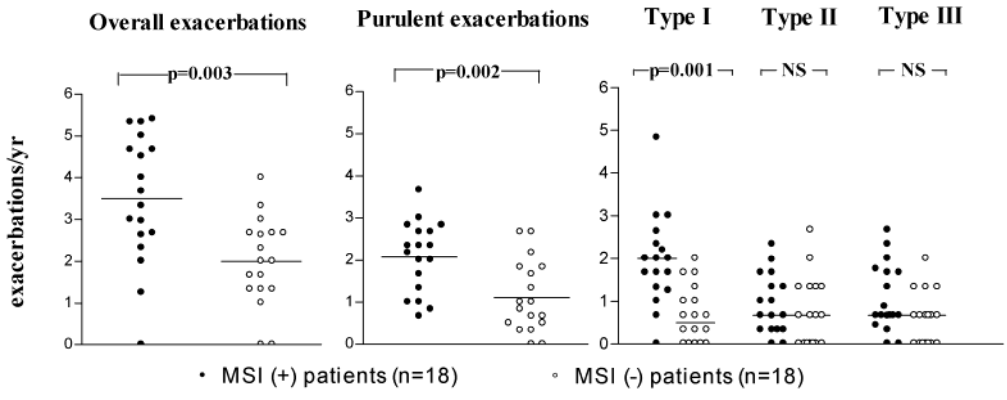
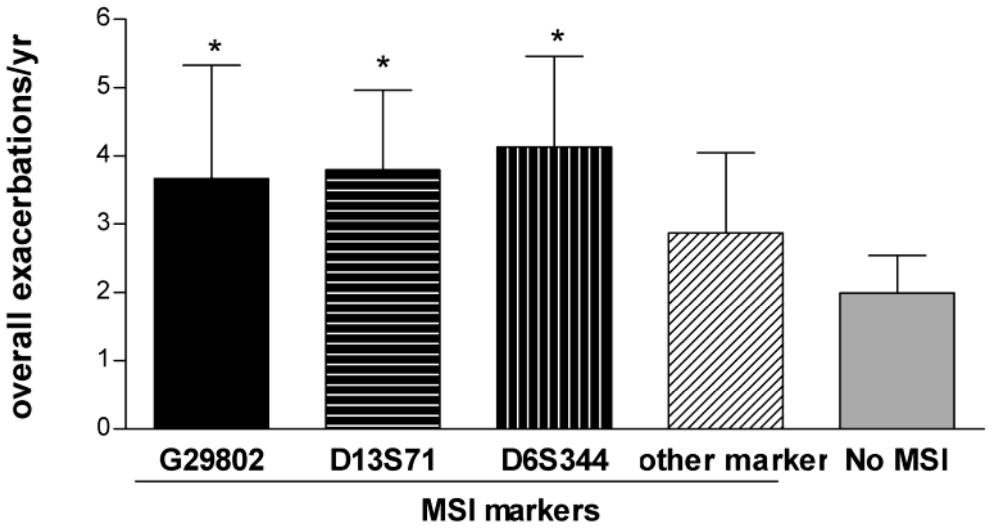
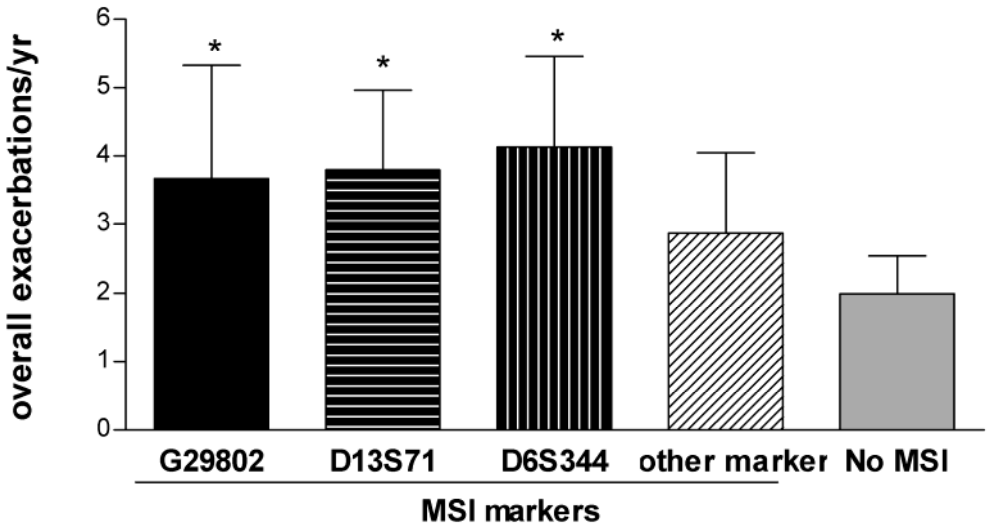



**Figure 4.** Individual annual exacerbation rates of COPD patients according to the presence of different Microsatellite DNA Instability (MSI) markers in their sputum. Horizontal lines represent median values.




* p<0.01




* p<0.01

**Table 1.** Baseline characteristics of 36 COPD patients by smoking status. Data are presented as Median (IQR) otherwise is indicated.

| | COPD patients | |
|---|---|---|
| | **Ex-smokers** | **Current smokers** |
| | **n=26** | **n=10** |
| **Age, yrs** | 69 (64-73)* | 61 (57-65) |
| **Male / female, n** | 24/2 | 8/2 |
| **BMI** | 25.5 (24-27) | 25 (23.5-27.5) |
| **$FEV_1$, %pred** | 41 (25-53) | 53.4 (51-68) |
| **Pack-yrs** | 45 (40-52) | 48 (38-55) |
| **$FEV_1$/FVC (%)** | 52.5 (41-63) | 54 (44-65) |
| **MRC score** | 2(0-4) | 1 (0-3) |
| **Bacterial colonization, n** | 9 | 1 |
| ***Induced sputum cells (%)*** | | |
| **Neutrophils** | 65.5 (61-67.5) | 64 (62-70) |
| **Macrophages** | 29.5 (25-34) | 29 (25-30) |
| **Lymphocytes** | 3.5 (2-4) | 2 (1.5-2.5) |
| **Eosinophiles** | 1.5 (1.2-1.8) | 2 (0-2) |
| **MSI positive subjects, n (%)** | 12 (46) | 6 (60) |

COPD = chronic obstructive pulmonary disease; BMI= Body Mass Index; $FEV_1$ = Forced Expiratory Volume in one second; FVC = Forced Vital Capacity; MRC= Medical Research Council. Continuous data are expressed as median(IQR) unless otherwise indicated; * p value between groups (Mann-Whitney test for continuous variables or chi-squared test for categorical data as appropriate).

**Table 2.** Cases exhibiting MSI according to each marker, the corresponding chromosomal location and related gene.

| Microsatellite marker | Chromosomal location | Related genes | Cases (n) with MSI |
|---|---|---|---|
| **RH70958** | 2p12 | -CD8 antigen, | 0 |
| **D5S207** | 5q31.3-q33.3 | -Interleukin 4 (IL-4)<br>-b2-adrenergic receptor | 1 |
| **D6S2223** | 6p21.3 | -Major histocompatibility (MHC)<br>-Tumor necrosis factor (TNF) | 0 |
| **D6S344** | 6p25 | - Protease inhibor 6 (PI6), Protease inhibitor 9 (PI9) | 5 |
| **D6S263** | 6p23-p24.2 | -Endothelin-1 | 3 |
| **G29802** | 10q22 | -Perforin 1 | 7 |
| **D13S71** | 13q32 | -Tumor necrosis factor ligand superfamily, member 13B | 8 |
| **D14S588** | 14q23-14q24 | -PTGDR (prostaglandin D2 receptor (DP)) | 5 |
| **D14S292** | 14q32.1 | -Constant region of heavy chain of IgE<br>-Alpha-1-antitrypsin<br>-Alpha-1-antichymotrypsin | 4 |
| **D17S250** | 17q11.2-q12 | -Apoptosis-antagonizing transcription factor<br>-Serotonin transporter<br>-Signal transducer and activator of transcription 5B (STAT5B). | 0 |
| Total | | | 33 |

**Table 3.** Exacerbation number and annual rates in all 36 COPD patients during the study period.

| | n | median (IQR) annual rate |
|---|---|---|
| **Overall Exacerbations** | 284 | 2.67 (1.67-3.42) |
| *Type I exacerbation* | 136 | 1.25 (0.70-2.15) |
| *Type II exacerbation* | 92 | 0.71 (0.41-1.42) |
| *Type III exacerbation* | 56 | 0.67 (0.11-1.33) |
| Purulent exacerbations | 172 | 1.67 (0.8-2.35) |

**Table 4.** Baseline characteristics of COPD patients according to Microsatellite DNA instability status (MSI positive or MSI negative).

| | **MSI (+) ve n=18** | **MSI (-) ve n=18** | **p** |
|---|---|---|---|
| **Age, yrs** | 68 (57-76) | 66.5 (59-70) | NS |
| **Female/male, n** | 1/17 | 3/15 | NS |
| **Current/ex-smokers, n** | 6/12 | 4/14 | NS |
| **Pack-years** | 47 (36-55) | 45 (35-55) | NS |
| **$FEV_1$, ml** | 1090 (735-1935) | 1215 (760-1665) | NS |
| **$FEV_1$ %pred** | 46 (22-58) | 47.5 (24-64) | NS |
| **$FEV_1$/FVC (%)** | 48 (41-67) | 58 (43-64) | NS |
| **MRC score** | 1 (1-3) | 1 (1-3) | NS |
| **Chronic cough, n(%)** | 8 (44) | 10 (55) | NS |
| **Chronic sputum, n(%)** | 7 (38) | 9 (50) | NS |
| **Bacterial colonization n(%)** | 5 (28) | 5 (28) | NS |
| ***Baseline treatment*** | | | |
| **Inhaled steroids** | 11 (61) | 12 (66) | NS |
| **Inhaled beta-agonists** | 13 (72) | 14 (77) | NS |
| **LTOT** | 3 (16) | 5 (27) | NS |

COPD = chronic obstructive pulmonary disease; $FEV_1$ = forced expiratory volume in one second. MRC= Medical Research Council; LTOT = Long-term Oxygen therapy. Continuous data are expressed as median(IQR) unless otherwise indicated; * p value between MSI(+) and MSI(-) subjects (Mann-Whitney test for continuous variables or chi-squared test for categorical data as appropriate).