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Viral Profile of COPD Exacerbations According to Patients[§]

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Abstract: *Background*: To compare the differences between elderly and non-elderly patients with acute exacerbations of chronic obstructive pulmonary disease (AECOPD) due to viral infections.

Methods: Patients with chronic obstructive pulmonary disease (COPD) exacerbation were recruited and classified as elderly (>65 years) and non-elderly (≤ 65 years). Sputum and oropharyngeal samples were assessed, PCR for respiratory viruses and cultures for common pathogens were performed.

Results: 247 patients (median age: 69.3±9.5 years) were recruited and categorized into group A: non-elderly patients [n=81 (32.8%), median age 58±5.99] and group B: elderly patients [n=166 (67.2%), median age 74.8±4.8] years. In 133 (53.8%) patients a viral infection was identified and in 34 (13.8%) a bacterial pathogen was isolated from cultures. In 18 (7.3%) patients a double infection (bacterial+viral) was identified. In group B, the presence of cardiac failure (46.6% *vs* 28.3%, p<0.001), renal failure (10.5% *vs* 4%, p=0.03), bacterial co-infection (13.8% *vs* 7.4%, p=0.04), influenza vaccination rates (45.5% *vs* 215, p<0.001), and longer hospital stay (8.4±4.4 *vs* 7.5±3.2 days, p=0.02) were higher than group A. The overall rate of viral infections did not differ according to age. A trend to higher rates of infection with parainfluenza 3 [19 (20%) patients in group B *vs*3 (7.5%) patients in group A, p=0.04] was observed in older patients.

Conclusion: No differences on the rate and type of viral infections were noted for elderly *vs* non elderly patients. However, they tended to have more bacterial co-infections that led to AECOPD and longer hospitalization stays compared to non-elderly patients.

Keywords: COPD exacerbations, elderly, PCR, viruses.

INTRODUCTION

The ageing process leads to a significant decline of physiologic and morphologic functions of the human body making the elderly patients more susceptible to infections [1]. The relationship between older age and infections cannot be easily evaluated since limited data exist concerning specific conditions (e.g. severe community acquired pneumonia, nosocomial bacteremia) or subpopulations (postoperative patients) within the elderly [2]. Up to date most of the efforts are small single center cohorts that have tried to study elderly patients together with specific characteristics and risk factors associated with certain infection [3-5].

Chronic Obstructive Pulmonary Disease (COPD) is complicated by frequent exacerbations, called Acute Exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD) which are associated with high morbidity, increased used of health resources and aggravation of the health status of the patients [6]. The main risk factor for AECOPD development is considered an infection of the respiratory system caused by a virus (up to 60%) or by bacteria (responsible approximately for 40% of those events) [7,8]. The significance of viral infections has been recognized during the last decade by the development and clinical application of newer molecular techniques able to detect viral species that are not easily detected by serology or viral cultures such as human Metapneumovirus [9]. Recently, we evaluated the epidemiology of viral infections in a cohort of patients with AECOPD where we showed that viral infections were strongly associated with AECOPD development independent of the stage of COPD and an infection with both a viral and a bacterial pathogen was common in these patients [10]. In this cohort, we had a high percentage of elderly patients with AECOPD due to viral infections. For this reason, we conducted the present study

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aiming to investigate possible differences among elderly and non-elderly patients with AECOPD of viral origin.

MATERIAL AND METHODS

Demographics

We have previously conducted a 2 year prospective study focused on the infectious etiology of AECOPD [10]. Briefly, in this cohort study, all patients (aged 40-90 years) who have visited the Emergency Department of a tertiary care hospital and were admitted into an inpatient facility because of a COPD exacerbation (according to GOLD criteria), without hospitalization during the last three months or use of antibiotics during the last 30 days and without a diagnosis of bronchial asthma were included [8]. The patients reported no history of infection during the last month and were not receiving systemic corticosteroid therapy. The study period lasted from January 2008 to June 2010. The study has taken approval by the ethical committee of the hospital and all the patients recruited in the study signed written informed consent. In the absence of a clear definition for "elderly" patient in the medical literature, the age cut off chosen for this study was based on the World Health Organization (WHO) definition for elderly. According to this statement most developed world countries have accepted the chronological age of 65 years as a definition of "elderly" or older person. While this definition is considered somewhat arbitrary, however it is usually associated with the age at which a person can begin to receive pension benefits. Currently, there is no United Nations standard numerical criterion, but the UN agreed to a cutoff of 60+ years in order to refer to the older population [11-14].

The following parameters were recorded on admission: age, sex, stage of COPD (according to GOLD criteria), smoking habits and alcohol addiction, use of oxygen, influenza vaccination, comorbidities (confirmed by medical records), current medication as well as signs and symptoms of respiratory tract infection. All patients underwent routine blood examination, including C- reactive protein, chest radiograph and sputum culture for common bacteria. The patients were assigned to COPD stages based on the GOLD criteria and according to the most recent available spirometric values (the last three months). Patients with mild COPD (stage I) had an FEV_1/FVC <70% and $FEV_1 \geq 80\%$ of predicted values. Patients with moderate COPD (stage II) had an FEV_1/FVC <70% and FEV_1 between 50% and 80% of predicted values. Patients with severe COPD (stage III) had an FEV_1/FVC <70% and FEV_1 between 30% and 50% of predicted values and patients with very severe COPD (stage IV) had an FEV_1/FVC <70% and FEV_1<30% of predicted values or FEV_1<50% of values predicted plus chronic respiratory failure. They all underwent spirometry after their discharge from hospital. All patients were followed-up throughout their recovery from exacerbation and until their discharge from the hospital.

"Microarrays Technique" [10]

Following first evaluation and before the initiation of any treatment, sputum and oropharyngeal samples (gargle) were collected and submitted for viral detection by clinical microarrays technique i.e. CLART® *PneumoVir* kit (GENOMICA, Spain). This method is able to detect and characterize the 17 most frequent types of human viruses responsible for respiratory infections, by identifying very small quantities of viral genomic material. It uses a sequence which corresponds to a highly preserved region within the viral genome and binding probes specific to each respiratory virus type. Viruses analyzed include, *human Respiratory Syncytial Virus* (hRSV) type A and B, *human Metapneumovirus* (hMPV) type A and B *Influenza* virus (all types A, B, C), *Rhinovirus*, *Parainfluenza* virus (hPIV) 1, 2, 3 and 4 (subtypes A and B), *Enterovirus* (Echovirus), *Adenovirus*, *Coronavirus* and *Bocavirus*. The samples were collected in Thin Prep CytoLyt® solution and centrifuged at 2000g. The molecular procedure was performed according to the manufacturer's instructions. In brief, viral DNA/RNA was extracted by using 200μl of clinical sample mixed with lysis buffer and then allowed to stand in room temperature for 15 minutes. Isopropanol was added and centrifugation was performed at 13000 rpm for 20 min followed by removal of the supernatant. Then the precipitate was resuspended with 1000μl 70% ethanol followed by centrifugation at 13000 rpm for 15 min. The supernatant was removed again and the sample was left to dry for 15 min (until there were no ethanol residues left). At the end the pellet was resuspended in 20 μl of dilution solution. The viral DNA/RNA extracts were stored at - 20°C until amplification. Virus amplification was performed *via* two RT (reverse transcriptase) Multiplex PCR reactions of a specific 120-330 bp fragment of the viral genome. The PCR employed the following thermal cycler settings: 1 cycle of 45 min at 45°C and 15 min at 95°C, followed by 45 cycles of 30 sec at 95°C, 1,5 min at 50°C and 1 min at 68°C and 1 final cycle of 10 min at 68°C. Visualization of the amplified product was performed on a platform based on low-density micro-arrays, which is called Array Tube (AT). This detection system is based on the precipitation of an insoluble product at those sites of the AT where the hybridization of the products amplified by specific molecular probes is produced. The amplified products are labeled with biotin during the PCR. After the amplification, they hybridize with their respective specific probes that are immobilized in specific and known sites of the AT, after which they are incubated with a streptavidin-peroxidase conjugate. The conjugate binds *via* streptavidin with the biotin present in the amplified products (which are also bound to their specific probes), while in the presence of o-dianisidine, the peroxidase activity of the conjugate induces the appearance of an insoluble product which precipitates at the hybridization sites of the AT" [10].

Statistical Analysis

Qualitative variables are expressed as counts and percentages and quantitative variables are expressed as means and standard deviations. The Student's t test for independent samples was used to perform comparisons between means whereas the Man-Whitney test was used in case variables did not meet the criteria of normality. The chi-square or Fisher's exact test were used for comparisons of proportions. A p value of less than 0.05 was considered

significant. Data were analyzed using statistical software (SPSS, version 15.0; SPSS; Chicago, IL).

RESULTS

In total, 370 patients were recruited in the study, 10 did not signed the inform consent, 80 did not meet the inclusion criteria while in 43 patients the samples were not suitable for analysis (we obtained an invalid result from the CLART Pneumovir®- the most common cause is that amplification process may have been interfered by an unknown substance which might inhibit the DNA polymerase enzyme). Altogether 247 patients were considered suitable to be analyzed [median age of 69.3±9.5 years old] and from them 81(32.8%) patients were ≤65 (58±5.99) years old and 166 (67.2%) ≥ 65 (74.8±4.8) years old (p<0.001). Demographics of all the recruited patients are shown in Table **1**. Elderly patients (group B) showed higher rates of vaccination against influenza (57% *vs* 21%, p<0.001), cardiac failure (55.4% *vs* 28.3%, p<0.001), renal failure (13.8% *vs* 4%, p=0.03) and bacterial (16.9% *vs*7.4%, p=0.04) infections. Among all recruited patients a viral infection was detected in 133(53.8%)] patients without however statistical differences between groups A and B [40(49.4) *vs* 93(69.9), p=0.3). The duration of hospital stay was longer in group B (8.9±4.8 *vs*7.5±3.2 days, p=0.02) without differences in mortality. Analyzing separately the 133 (53.8%) patients where a virus was isolated we found that the age, the vaccination rate against influenza [56(63.4%) *vs* 5(12.5%), p<0.001] and the use of inhaled steroids [68(73.1%) *vs* 19(47.5%), p=0.008) were higher in group B (Table **2**).

The detected viruses in order of frequency were human Respiratory Syncytial Virus (hRSV) subtypes A and B [n= 108 (81.2%)], influenza virus type A, B and C [n=34 (25.5%)], parainfluenza virus (hPIV) sybtypes 3 and 4 [n=26 (19.5%)], rhinovirus [n=17 (12.8%)], human Metapneumovirus (hMPV) subtypes A and B [n=10 (7.5%)], coronavirus [n=5 (3.7%)], Adenovirus [4 (3%)], Enterovirus

Table 1. All patients with AECOPD.

Demographics n (%)	All Patients	Patients ≤65 Years Group A	Patients >65 Years Group B	*p* Value
Number of patients	247	81(33%)	166 (67%)	<0.001
Age (years)	69.3±9.5	58 ±5.99	74.8±4.8	<0.001
Sex (Males)	191 (77.3)	59 (72.8)	132 (79.5)	0.2
Oxygen therapy	112 (45.3)	27 (33.3)	85 (51.2)	0.08
Influenza vaccination	112 (45.3)	17 (21)	95 (57)	<0.001
Pack years	62±40.66	61.6±39.02	62.1±41.5	0.6
COPD (years)	6.2±6.37	4.7±5.07	7.04±6.7	0.007
FEV1 (%pred)	44.6±16.7	46.18±17.9	43.67±16.21	0.32
Inhaled steroids	156 (63.2)	43 (53)	112 (67.5)	0.02
Comorbidities n (%)				
Cardiac failure	115 (46.6)	23 (28.3)	92 (55.4)	<0.001
Renal Failure	26 (10.5)	3 (4)	23 (13.8)	0.03
Diabetes	68 (27.5)	16 (20)	52 (31)	0.056
Laboratory findings				
$PO_{2\,(}$mmHg)	58.1±12.4	59.5±10.6	57.5±13.2	0.07
PCO_2 (mmHg)	46.5±15.3	45.8±15.3	46.8±15.4	0.4
pH	7.4±0.06	7.41±0.05	7.4±0.06	0.1
WBC(c/mm^3 x 10^3)	11.1±7.6	11.4±11.5	11±4.8	0.6
CRP (mg/dl)	3.7±5.6	2.7±3.8	4.1±6.2	0.06
Microbiology				
Infection with a virus	133 (53.8)	40 (49.4)	93 (69.9)	0.3
Infection with a bacteria	34 (13.8)	6 (7.4)	28 (16.9)	0.04
Length of stay (days)	8.4±4.4	7.5±3.2	8.9±4.8	0.02
Outcome				
Survived	234 (94.7)	78 (96.3)	156 (93.9)	0.4
Non - Survived	13 (5.3)	3 (3.7)	10 (6)	0.4

AECOPD= Acute Exacerbation Chronic Obstructive Pulmonary Disease, CRP== C-Reactive Protein, WBC=White Blood Cells.

Table 2. Elderly *vs* non-elderly patients with AECOPD with a viral infection.

Number of Patients N=133 (100%)	Patients ≤ 65 Years N=40(30%)	Patients >65 Years N=93(70%)	*p* Values
Age (years)	57.5±5.7	75.3±5.1	<0.001
Sex (Males) (%)	26 (65)	71 (76)	0.2
Oxygen therapy (%)	12(30)	50(53.7)	0.05
Influenza vaccination (%)	5(12.5)	56(63.4)	<0.001
Pack years	58.8±42.6	55.2±38.8	0.4
COPD (years)	4.7±5.4	7.5±7	0.1
FEV1	47.6±16.	46.8±16.8	0.6
Inhaled steroids	19(47.5)	68 (73.1)	0.008
Comorbidities			
Cardiac failure (%)	11(27.5)	52(55.9)	0.05
Renal failure (%)	2 (5)	14(15)	0.1
Diabetes (%)	8(20)	29(31)	0.2
Laboratory findings			
PO2 (mmHg)	59.6±11.5	57.3±13.5	0.4
PCO2 (mmHg)	46.3±13.5	45.7±13.9	0.7
pH	7.41 ± 0.05	7.42±0.7	0.01
WBC(c/mm3x 103)	9.6±2.9	10.8±4.5	0.056
CRP (mg/dl)	2.6±3.3	4.4±6.3	0.09
Microbiology			
Bacterial infection (%)	2(5)	16(17.2)	0.1
Length of stay (days)	7.78±2.6	9.84±5.05	0.01
Outcome			
Survived	38(95)	89(95)	0.7
Non-Survived	2(5)	3(3)	0.9

AECOPD= Acute Exacerbation Chronic Obstructive Pulmonary Disease, CRP== C-Reactive Protein, WBC=White Blood Cells.

[n=1 (0.7%)], Echovirus [n=1 (0.7%)] and Bocavirus [n=1 (0.7%)]. The type of isolated viruses according to the age is shown in Fig. (**1**) and Table **3**. No statistically significant differences were noted in any of the two groups with the exception of hPIV3 infection [19 (20%) patients in group B *vs*3 (7.5%) patients in group A, p=0.04]. Also mixed viral infections [63 (47.3%)] (isolation of more than two viruses) presented the same frequency in elderly and in non-elderly group [45 (48%) patients for group B *vs* 18 (45%) patients for group A, p=0.08] and the same observation was made for influenza C infection [11 (12%) patients in group B *vs* 1 (2.5%) patients in group A, p=0.06]. In 34 (13.8%) patients a bacterial infection and in 18 (7.3%) patients an infection with both a bacterial and a viral pathogen (dual infection) were identified. The most commonly isolated bacteria were *Pseudomonas aeruginosa, Haemophilus influenzae, Klebsiella pneumoniae, Staphylococcus aureus, Acinetobacter baumanii* and *Stenotrophomonas maltophilia.*

Statistically significant difference was evident in the seasonal pattern of RSV infection (both subtypes-p=0.001, p=0.029), hPIV3 (p=0.003), hPIV4 infection (p<0.001) and influenza virus infection (all the subtypes) (p=0.009, p= 0.023, p=0.049 respectively) (Table **4**), without however a statistically difference between elderly and non-elderly patients (Table **5**).

DISCUSSION

In this study we found: a) a high frequency of AECOPD due to viral infections in elderly and non-elderly patients without differences between the two groups, b) more frequent infections due to human *Parainfluenza* virus (hPIV) and influenza in elderly patients compared to non-elderly longer and c) lengthier hospital stays for the elderly patients.

In a recently published study where we evaluated the epidemiology of viral infections in patients with AECOPD a high rate of viral infections (53.8%) was detected which was in accordance with other previously published reports [7,8,10]. In this study when comparing the frequency of viral infections among elderly and non-elderly patients no differences were detected. A possible explanation for this could be the fact that COPD is a chronic systemic inflammatory syndrome affecting the immune response independently of the age and making these patients more prone to such infections [15,16]. The elderly is a large and even growing population, proportional to the age of the

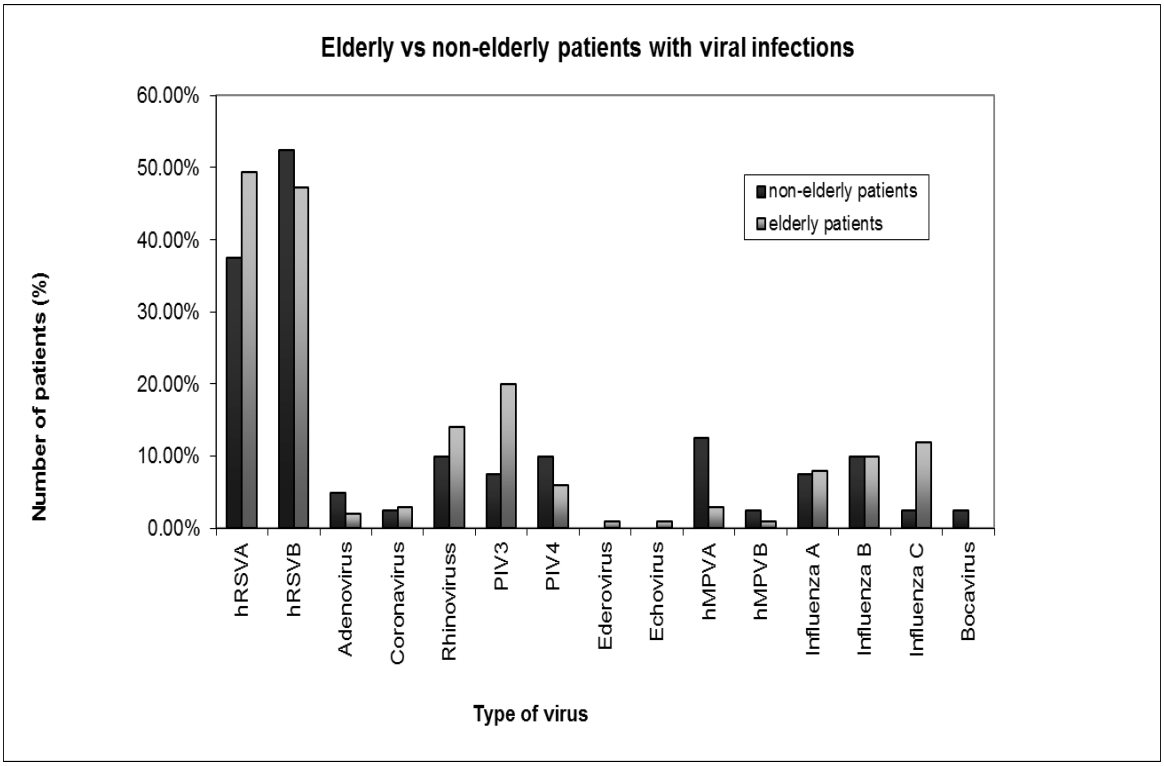

Fig. (1). Elderly *vs* non-elderly patients with viral infections.

Table 3. Elderly *vs* non-elderly patients with AECOPD : isolated viruses.

Type of Virus	Number of Isolated Viruses in N=133 (100%) Patients	Viruses Isolated in Patients ≤ 65 Yrs in N=40 (30%) Patients	Viruses Isolated in Patients > 65 Yrs in N=93 (70%) Patients	*p* Values
hRSVA	61	15	46	0.1
hRSVB	65	21	44	0.9
hRSV total	108	32	76	0.9
Influenza A	11	3	8	0.6
Influenza B	14	4	10	0.7
Influenza C	12	1	11	0.06
Influenza total	34	9	25	0.7
Rhinovirus	17	4	13	0.3
hPIV3	22	3	19	0.04
hPIV4	10	4	6	0.6
hPIV total	26	5	21	0.3
Coronavirus	4	1	3	0.7
hMPVA	8	5	3	0.7
hMPVB	2	1	1	0.6
hMPV total	10	4	6	0.6
Adenovirus	4	2	2	0.4
Echovirus	1	0	1	0.4
Enterovirus B	1	0	1	0.4
Bocavirus	1	1	0	0.1
Double virus infection	63	18	45	0.08

hRSVA, B =human Respiratory Suncytial Virus A and B, hPIV3,4=human Parainfluenza Virus type 3, 4= human Parainfluenza Virus type 4, hMPVA, B= human Metapneumovirus type A, B, $p<0.05$ is considered statistical significant.

Table 4. Seasonal pattern of respiratory viruses.

Season	Winter	Spring	Summer	Autumn	*p* Value
Number of Patients	**N=71 (28.7%)**	**N=81 (32.8%)**	**N=21 (11.3%)**	**N=67 (27.1%)**	
hRSVA	25 (35.2)	17 (21)	12 (42.9)	7 (10.4)	0.001
hRSVB	11 (15.5)	20 (24.7)	11 (39.3)	23 (34.3)	0.029
Adenovirus	2 (2.8)	2 (2.5)	0	0	0.46
Coronavirus	1 (1.4)	3 (3.7)	0	0	0.28
Rhinovirus	2 (2.8)	4 (4.9)	3 (10.7)	8 (11.9)	0.13
hPIV3	2 (2.8)	4 (4.9)	3 (10.7)	13 (19.4)	0.003
hPIV4	1 (1.4)	0	0	9 (13.4)	<0.001
Enterovirus B	0	1 (1.2)	0	0	0.5
Echovirus	0	1 (1.2)	0	0	0.5
hMPVA	3 (4.2)	4 (4.9)	0	1 (1.5)	0.46
hMPVB	1 (1.4)	1 (1.2)	0	0	0.7
Influenza A	8 (11.3)	2 (2.5)	1 (3.6)	0	0.009
Influenza B	3 (4.2)	7 (8.6)	4 (14.3)	0	0.023
Influenza C	0	2 (2.5)	0	10 (14.9)	<0.001
Bocavirus	0	0	1	0	0.05
Double viral infection	**18 (25.3)**	**16 (19.7)**	**9 (32.1)**	**20 (29.9)**	**0.5**

hRSVA, B =human Respiratory Suncytial Virus A and B, hPIV3,4=human Parainfluenza Virus type 3, 4= human Parainfluenza Virus type 4, hMPVA, B= human Metapneumovirus type A.

general hospitalized population. This group of patients is characterized by a decline of the immunological response to infection, principally due to functional insufficiency of monocytes and macrophages that results to inadequate phagocytosis, by the lack of antigen presenting cells, such as dendritic cells (so are naive T-cells due to thymus gland involution), by the loss of memory capacity of mature T-cells exhibiting a poor and/or altered cytokine production and by the decrease of the number of circulating B-cells resulting in a weaker response to antigenic challenges through immunoglobulin production [17,18]. Elderly usually have higher rates of vaccination against influenza from their primary care physicians than non-elderly (although both are at risk) [15,16]. However, no difference was detected either in influenza virus or other viruses' detection rates. This could be explained by the fact that immune responses to vaccination decline substantially with age thus the elderly have impaired humoral and cell mediated immune responses to influenza vaccines compared with younger adults [13,14,19-21]. It also points to the need for better prevention measures against respiratory viruses for this population.

A strong association between comorbidities (number and type) and older age is well known [22].

COPD patients usually have increased number of comorbitities (cardiovascular diseases, respiratory tract diseases, metabolic diseases, haematological diseases / coagulopathies, musculoskeletal diseases, gastro-intestinal diseases, renal diseases, psychiatric diseases, neoplasias) known as "COPD comorbidome" which are considered as COPD- related (e.g. respiratory failure, pulmonary heart disease cachexia) or COPD-non related (eg obesity, diabetes mellitus, arterial hypertension) [23-25]. In our patients cardiac/ renal failure and diabetes mellitus were the most frequent detected comorbitities. This increased number could be explained by the orientation of our center as is a specialized site on cardio-respiratory diseases in Greece.

The infections due a bacterial pathogen were more common in elderly subjects and the hospital stay was lengthier. These patients need more time to recover because of the complexity of COPD (either a systemic oxidative stress syndrome or an inflammatory process or a combination of those), the weaker immune response as result of the inflammatory process and the frequent colonization by bacteria that lead to bacterial infections or co-infections (viral+bacterial) [22,26-31]. This is a main statement in a recently published study where a longer hospital stay in AECOPD patients with co-existed candidiasis, anemia, psychological disorder, atrial fibrillation and congestive heart disease, asthma, respiratory failure and cachexia was detected [24]. We didn't find any difference in the mortality in elderly AECOPD patients with a viral infection compared to the non-elderly although mortality is higher in COPD patients with lung cancer, pulmonary heart disease, heart failure, atrial fibrillation, obstructive sleep apnea, obesity, osteoporosis and asthma [24].

The isolated type of viruses in two groups didn't present any difference (Table **3**) except hPIV3 detection. Human parainfluenza viruses (hPIVs) are considered to be one of the most common causes of lower respiratory tract infections in children [32-34] but it is difficult to understand their biologic significance in this cohort. One possible explanation could have been the occurrence of a clonal hPIV outbreak during

Table 5. Seasonal pattern of respiratory viruses in non-elderly and elderly patients.

Season	Winter (%)			Spring (%)			Summer (%)			Autumn (%)		
Isolated Viruses	**NE=13**	**E=31**	**P value**	**NE=11**	**E=31**	**P Value**	**NE=5**	**E=10**	**P Value**	**NE=11**	**E=24**	**p Value**
hRSVA	6 (46)	19 (61)	0.56	4 (36)	13 (42)	0.75	3 (60)	9 (90)	0.49	2 (18)	5 (20)	0.74
hRSVB	4 (30)	7 (13)	0.36	6 (54)	14 (45)	0.87	4 (80)	7 (70)	0.83	7 (63)	16 (67)	0.87
Adenovirus	1 (7)	1 (3)	0.8	1 (9)	1 (3)	0.98	0	0	-	0	0	-
Coronavirus	0	1 (3)	0.6	1 (9)	2 (6)	0.72	0	0	-	0	0	-
Rhinovirus	0	2 (6)	0.9	2 (18)	2 (6)	0.56	0	3 (30)	0.49	2 (18)	6 (25)	0.98
hPIV3	0	2 (6)	0.9	0	4 (13)	0.5	0	3 (30)	0.49	3 (27)	10 (41)	0.67
hPIV4	0	1 (3)	0.6	0	0	-	0	0	-	4 (36)	5 (21)	0.59
Ederovirus B	0	0	-	0	1 (3)	0.54	0	0	-	0	0	-
Echovirus	0	0	-	0	1 (3)	0.54	0	0	-	0	0	-
hMPVA	2 (15)	1 (3)	0.4	1 (9)	3 (9.5)	0.58	0	0	-	0	1 (4)	0.65
hMPVB	0	1 (3)	0.6	0	1 (3)	0.54	0	0	-	0	0	-
influenza A	3 (23)	5 (16)	0.9	0	2 (6)	0.98	0	1 (10)	0.71	0	0	-
influenza B	0	3 (9.5)	0.6	2 (18)	5 (16)	0.75	2 (40)	2 (20)	0.83	0	0	-
influenza C	0	0	-	0	2 (6)	0.98	0	0	-	1 (9)	9 (37.5)	0.18
Bocavirus	0	0	-	0	0	-	1(20)	0	0.71	0	0	-

hRSVA, B =human Respiratory Suncytial Virus A and B, hPIV3,4=human Parainfluenza Virus type 3, 4= human Parainfluenza Virus type 4, hMPVA, B= human Metapneumovirus type A, B, NE=non=elderly, E=Elderly.

the study period. When analyzing the seasonal pattern of viral infections no statistically differences were noted although is well known, that COPD patients present more commonly exacerbations during the cold seasons (winter-autumn) and viral infections have a higher prevalence during this period associated with a longer recovery period, longer in house stay and increased likelihood of hospital admission [12,35]. Also, despite the important role of viral infections, in this study it was difficult to examine their true pathogenic role since for some of these viruses there is an increasing evidence that they could colonize the respiratory tract; such colonizers may act as modulators of the local immune response in a subsequent bacterial upper tract infection in an already susceptible patient but the exact interaction of bacterial with viral colonizers is an issue for further debate [36, 37].

This study has some limitations. First, no quantitative PCR techniques were performed in order to test the modifications of the viral load of a specific virus which could be indicatives of the (re)activation of the virus in a previously colonized patient before the exacerbation. Second, patients with AECOPD frequently receive home care for moderate- mild episodes and for this reason we could have missed a fraction of similar episodes occurring in the community. Third, because of the low yield of sputum cultures regarding the confirmation of bacterial infections we could have missed some bacterial pathogens inhabiting or infecting the respiratory tract. Forth, there is no evident a convinced explanation for the high detection of RSV.

In conclusion, in a significant percentage of elderly people with AECOPD a viral pathogen was detected in their upper respiratory tract. Human parainfluenza viruses and mixed viral infections were more common in elderly subjects but the exact role of the different viral species is a matter for further research.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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