Demet KARNAK[1]
Sumru BEDER[1]
Oya KAYACAN[1]
Levent KARACA[2]

IgG Subclass Levels in ELF in COPD

Abstract: Objective: Deficiency of immunoglobulin (Ig) may be responsible for recurrent infections in chronic obstructive pulmonary disease (COPD). Our aim was to investigate the level of IgG subclass (IgGsc) in the epithelial lining fluid (ELF) and to try to find a possible relation between these levels and serum or bronchoalveolar lavage (BAL) IgGsc levels in COPD patients with recurrent acute exacerbation.

Methods: Twenty-four clinically stable non-smoking COPD patients who had developed recurrent exacerbations three or four times a year and 17 individuals without COPD were enrolled as the experimental and control groups (Group I and II) respectively. The BAL procedure was performed on the groups by bronchofiberoscope. In order to calculate ELF Ig values, IgGsc and urea levels in sera and BAL fluids were measured.

Results: Total Ig levels did not differ between the groups. All serum IgGscs except IgG1 increased in Group I versus the control group [mean values (mg/L)]: IgG2=3734, IgG3=1119, IgG4=405; p<0.05). However, in the ELF, mean IgG1 and IgG2 levels significantly decreased (367.3mg/L and 201.7mg/L respectively; p<0.05). BAL IgGsc/urea ratios significantly were correlated with ELF IgGsc levels (p<0.0001, r>0.8) suggesting that only BAL urea and IgGsc measurements were sufficient when comparing the groups.

Conclusion: Immunologic response may be impaired in COPD due to the deficiency of IgGsc. The decrease in IgG1 and IgG2 in ELF, which impairs the local pulmonary response, may be responsible for the recurrent exacerbations in patients with COPD.

Key Words: Bronchoalveolar lavage (BAL), epithelial lining fluid (ELF), chronic obstructive pulmonary disease (COPD), immunoglobulin deficiency, immunoglobulin subclass deficiency.

Departments of [1]Chest Diseases and Tuberculosis, [2]Biochemistry, Faculty of Medicine, Ankara University, Ankara - TURKEY

Introduction

Deficiency of immunoglobulin(s) (Ig) in serum is characterized by recurrent infections such as bronchitis, sinusitis and otitis media, usually caused by *S. pneumoniae, H. influenzae* and *S. aureus*. For these patients, deficiency of immunoglobulin A (IgA) may co-exist (1). Defects in the immune system have recently been reported in patients with chronic obstructive pulmonary disease (COPD). The most frequently detected defect is the humoral immune defect. The deficiency of IgG subclass (IgGsc) has been implicated for the recurrent infections in patients with COPD (2,3).

For acutely exacerbated COPD patients, the number of bacteria in the sputum is higher than during the stable period, and/or neutrophil leukocytes are two-fold elevated. In contrast to other subjects with infection, these patients do not necessarily have fever, chills, leucocytosis or a high erythrocyte sedimentation rate. Before starting the treatment for an acute exacerbation, the causative infective agent should be determined. Some of the most common causative agents are *S. pneumoniae*, *H. influenzae*, *Morexella catarrhalis* and viruses (4).

The major soluble Igs in bronchoalveolar lavage (BAL) fluid are IgGsc and IgA. A small amount of IgM is also found. Complements acting in classical and alternate pathways can also be shown in BAL fluid (5-9).

IgG constitutes of 70-75% of serum Igs. It has four subclasses, namely IgG1, IgG2, IgG3 and IgG4 (10-12). IgG1 and IgG3 are complement fixating antibodies, whereas IgG2 and IgG4 can bind to a complement weakly. IgG1, IgG2 and IgG4 are responsible for the immune reaction against the protein antigens. IgG4 is a blocking antibody like IgE, and it can initiate type I and type III allergic responses (13-15).

Since IgG1 constitutes the major part of total IgG, its deficiency gives rise to serious clinical problems such as sepsis or necrotizing enterocolitis in infancy or recurrent sinopulmonary infections in early childhood. In the deficiency of IgG2, sinopulmonary infections are frequently seen and these patients cannot cope with bacteria which have a polysaccharide cell wall. Isolated or combined deficiency of IgG3 is rarely seen and may be familial. IgG4 may play a role in asthma and is too low to be detected in serum or in BAL, unless commercial kits for ultralow levels are used (10,11,13,16,17).

Our aim was to investigate the levels of Igs and IgGscs in epithelial lining fluid (ELF) of COPD patients with recurrent lower respiratory tract infections.

Materials and Methods

Study subjects

Forty-one patients (33M, 8F) were enrolled in the study. The mean age was 50.2±17.2 years, ranging from 17 to 73.

Group I: Twenty-four patients (20 males, 4 females) with COPD, who were clinically stable for at least 6 weeks, constituted Group I. The diagnosis of COPD was established as defined by the American Thoracic Society (ATS) (4). The mean age was 60.1±10.4 years, ranging from 30 to 73. They were not on systemic steroids and they were ex-smokers for at least one year with a history of about 49 pack-years of smoking. All patients had a history of acute exacerbation at least three or four times a year. The patients with acute exacerbation were given treatment until they were in stable clinical condition lasting at least 6 weeks. Patients who had bronchiectasis, sinusitis, tonsillitis or any other chronic infective diseases or malignancy were excluded.

Group II: Seventeen control patients (13 males, 4 females) with a history of tobacco consumption of about 30 pack-years constituted the control group. The mean age was 56.1±14.7 years, ranging from 17 to 61. They underwent Fiberoptic bronchoscopy (FOB) for indications as follows: nonproductive cough (n:7), hemoptysis workup (n: 3), suspected tuberculosis activity (n: 2), suspected hilar or pulmonary mass (n: 2 in each) and suspected sarcoidosis (n: 1). Although FOB indications suggest some disease, in Group II patients, followed up for eight or twelve months, no malignant disease or disease suspected before FOB was observed. Furthermore, they had not used any drug or smoked for at least one year and had no symptoms of COPD.

Study design: Prospective study performed in a university hospital. The independent variable was existence of COPD with frequent acute exacerbations and the dependent variable was deficiency of immunoglobulins in ELF and related samples.

The study was approved by the Ankara University Hospital Ethics Committee and written informed consent was obtained from each subject before their participation in the study.

FOB: The tracheobronchial tree was observed through a bronchofiberoscope (Olympus BF, IT20D). The bronchial mucosa was visually evaluated by two bronchoscopists in the same session and the chronic mucosal changes due to inflammation were scored by using the criteria of chronic bronchitis index (CBI) (18)(Table 1). Special attention was paid to the relation between CBI and clinical symptoms. The bronchoscopists were unaware of the study group and they were also unaware of the score of each other.

BAL: The bronchofiberoscope was wedged in the peripheral bronchus of the middle lobe or lingula and a catheter was pushed out of the distal end. Then 100 ml of 0.9% saline solution was instilled in 5 aliquots and retrieved immediately with "zero dwell time". As BAL measurements are important for ELF calculations, "dwell time" was assumed to be less than 20 seconds. Increased dwell time is known to result in increased concentrations of urea and protein level in the recovered BAL. Immediate aspiration of instilled saline allowed the least possible amount of diffussion of molecules from sources. Using a time of 20 seconds, this calculation suggested that a minimum of 80% of urea recovered in BAL fluid was derived in situ in ELF and a maximum of 20% was derived from diffusion. By this procedure approximately 50±2% of the 100 ml of instilled saline was recovered in both groups. The BAL was filtered through 3 layers of gauze and centrifuged at 500 g for 10 minutes. The supernatant was stored at –70°C for Ig analysis and at –20°C for urea analysis (19,20).

Immunoglobulin analysis: Serum IgG, IgA, IgM and IgE levels were measured with nephelometry in our laboratory of immunology. Normal range of values were,

Table 1.   Endoscopic scores of airways inflammation (CBI)

Score	Erythema	Oedema	Secretions	Friability
0	None	None	None	None
1	Light red	Blunting of airway bifurcations	Strands of clear mucus	Punctate submucosal haemor rhage with scope trauma
2	Red	Loss of normal airway indentations	Globules of mucus	Linear submucosal haemor rhage with scope trauma
3	Beefy red	Airway occluded	Airway occluded	Frank bleeding with scope trauma

IgG: 6.5-16 g/L; **IgA:** 0.45-3.5 g/L; **IgM:** 0.5-3.2 g/L; **IgE:** 1.0-100 kU/ml.

Quantitative IgG subclass measurements in sera (s-IgGsc) were performed using a radial immunodiffusion (RID) kit (Single Dilution RID, BIND A RID-The Binding Site Limited-UK). The control serum in the kit was used for quality control purposes. This serum was treated exactly like a test sample, giving a diameter in keeping with the concentration stated on the bottle, confidence limits ±0.3mm. For example, if the IgG1 concentration quoted on the serum bottle was 6100 mg/L, this was equivalent to a ring diameter of 6.4mm (from RID reference table). The control serum therefore gave a ring diameter in the range 6.1-6.7mm and normal ranges of this kit were (g/L),

IgG1: 3.15-8.42; IgG2: 1.39-5.54; IgG3: 0.04-1.19; IgG4: 0.01-0.9 (21).

Levels of IgGsc in the BAL (B-IgGsc) of the subjects were measured with commercial kits (Specific Instructions IgG Subclasses Ultra Low Level- NANORID-The Binding Site Limited-UK). After the required diffusion time, ring diameters were measured to the nearest 0.1mm with a jewellers' eyepiece in a double blind manner for serum and B-IgGsc measurements. For BAL fluid samples suspected of containing low concentrations of the specific protein, a "double fill" of the well was performed, along with the 1:1 diluted calibrator of the respective low value, and the obtained results were corrected (21-23).

IgGsc content of ELF: As BAL is a diluted material representative of the lower respiratory tract, ELF was also worked out. Because of its ready diffusion through the body compartments including the lung, urea concentrations in sera and lavage fluids were used to quantify the IgGsc concentrations of ELF. The concentrations of urea in serum and in situ ELF are assumed to be identical. The level of IgGsc in ELF (E-IgGsc) was worked out using the following formulas (19,24-27):

$$(1)\ \text{ELF volume} = \frac{\text{BAL[urea] x BAL volume}}{\text{Serum [urea]}}$$

$$(2)\ \text{E-IgGsc} = \frac{\text{BAL volume x B-IgGsc}}{\text{ELF volume}}$$

$$(3)\ \text{E-IgGsc} = \frac{\text{B-IgGsc x Serum [urea]}}{\text{BAL [urea]}}$$

To measure urea concentration in BAL fluid, a commercially available serum urea measuring kit (Sigma Diagnostics, BUN, Endpoint, UV-66) was used with slight modifications. The BUN concentration is measured by coupled enzyme reactions involving urease and glutamate dehydrogenase (coefficient of variation: 0.6-2.4%). In contrast to the 3.0 ml recommended by the supplier, a 2.5 ml reaction mixture was used and a 0.5 ml BAL fluid sample (rather than the 0.01 ml in the instructions) was analyzed in combination with saline and lavage fluid blanks to correct for dilution reactant and optical absorbance of the sample at 340 nm respectively (20). In a recent study by Wichert et al., urea concentrations were less variable than albumin concentrations in patients from different disease groups, and it was concluded that urea might be the preferred denominator for BAL studies (28).

Pulmonary Function Test (PFT): Spirometric tests were performed using Vitalograph alpha spirometer. PFT parameters were expressed as percentages of the predicted values.

Arterial blood gas analysis: The arterial blood gases were analyzed by Radiometer ABL 330. PaO2 and PaCO2 were expressed in mmHg, and oxygen saturation in percentages.

Statistics: Statistical evaluation was done with a PC using SPSS. The results were expressed as mean ± standard deviation (SD). The differences between the groups were evaluated by Student's t test and Mann-Whitney U test. The correlation of B-IgGsc and E-IgGsc was assessed by the Spearman correlation analysis test. Any p value less than 0.05 was considered significant.

Results

Both groups had smoked almost equally but the spirometric results and arterial blood gas analysis of Group I were significantly deteriorated as expected and the visual endoscopic scores for the inflammation of the bronchial mucosa (CBI) were significantly elevated when compared to those of Group II (p<0.01)(Table 2).

Both groups were comparable in terms of total Ig main class levels in the sera. We did not find IgGsc or main class deficiency in sera between the groups. According to normal ranges, 66% of Group I patients had higher than normal values of at least one IgGsc. When mean levels of Igs were compared, the main class of Igs (IgG, IgM, IgA) was not different between the groups. Although IgE levels of eight subjects (38%) of Group I and three (33%) of Group II were high, the groups did not significantly differ (p>0.1) (Table 3).

In Group I, mean levels of IgG2, IgG3 and IgG4 in sera were found to be significantly high (p<0.05). However, IgG1 and IgG2 in ELF (or BAL) were significantly lower than those in Group II (p<0.05) (Table 4).

In order to standardize the B-IgGsc results, we planned to express them as ratios of B-IgGsc to urea (Table 4). As B-IgGsc/urea ratios were strongly correlated with E-IgGsc values (r>0.8, p<0.001) (Figure 1), we expressed the value of B-IgSC as ELF Igs by applying the formulas mentioned above.

Discussion

Groups I and II were comparable in terms of smoking history. However, Group I had lower spirometric PaO_2 and O_2 saturation values, and higher $PaCO_2$ and CBI values when compared to Group II. These findings were all expected because the control subjects had normal lungs in clinical and radiological aspects although they had smoked as heavily as Group I had.

In Group I, mean s-IgGsc (IgG2, IgG3, IgG4) levels were significantly elevated. Sixteen subjects of Group I

	Group I (COPD) (mean±SD)	Group II (CONTROL) (mean±SD)	p
Age (years)	60.2±10.4	56.1±14.9	p>0.05
Smoking history (pack-years)	49.2±30	34 ±23	p>0.05
FVC (% of predicted)	70.7±27.9	83.9±29.9	p>0.05
FEV1 (% of predicted)	62.9±31.2	92.6±19.3	p=0.003
FEV1/FVC (%)	64±19.1	75.9±20.9	p=0.004
MMF (% of predicted)	37.1±36.7	72.2±39.2	p=0.006
PaO2 (mmHg)	64.9±13.3	83.7±9	p=0.0001
PaCO2 (mmHg)	39.2±6.9	32.2±3.6	p=0.005
CBI	18.5±9.9	6.7±3.6	p=0.003

Table 2. Features of the subjects

Table 3. Comparison of serum Ig main classes in both groups. Serum Igs were not statistically different between the groups.

	IgG (g/L)	IgM (g/L)	IgA (g/L)	IgE (kU/m/L)
Group I (COPD)	18.24±6.10	2.71±3.57	3.22±2.33	327±605.8
Group II (CONTROL)	16.52±3.15	2.80±2.08	2.30±0.33	138.20±239

Table 4.   Comparison of serum and ELF IgGsc levels in COPD and control subjects.

| Immunoglobulins (mg/L) (mean±SD)	Location	Group I (COPD) (n=24)	Group II (CONTROL) (n=17)	p value
IgG1	Serum	6714.17± 2373.14	6625.88± 1809.46	p>0.05
	BAL IgG1/urea	2.65±3.67	6.58±7.00	**p=0.01**
	ELF	367.38± 325.71	1019.8±1264.15	**p=0.01**
IgG2	Serum	3734.17± 1356.30	2954.12± 1106. 52	**p=0.04**
	BAL IgG2/urea	1.25±1.94	2.13±1.89	**p=0.03**
	ELF	201.76±245.35	360.5± 435.76	**p=0.04**
IgG3	Serum	1119.21± 322.67	728.06± 289.53	**p=0.0007**
	BAL IgG3/urea	0.64±0.49	1.01±0.84	p=0.05
	ELF	114.9±113.64	167.02± 188.2	p>0.05
IgG4	Serum	405.79± 182.43	284.41± 169.44	**p=0.02**
	BAL IgG4/urea	0.33±0.42	0.51±0.45	p=0.05
	ELF	74.0± 133.9	78.7± 77.9	p>0.05
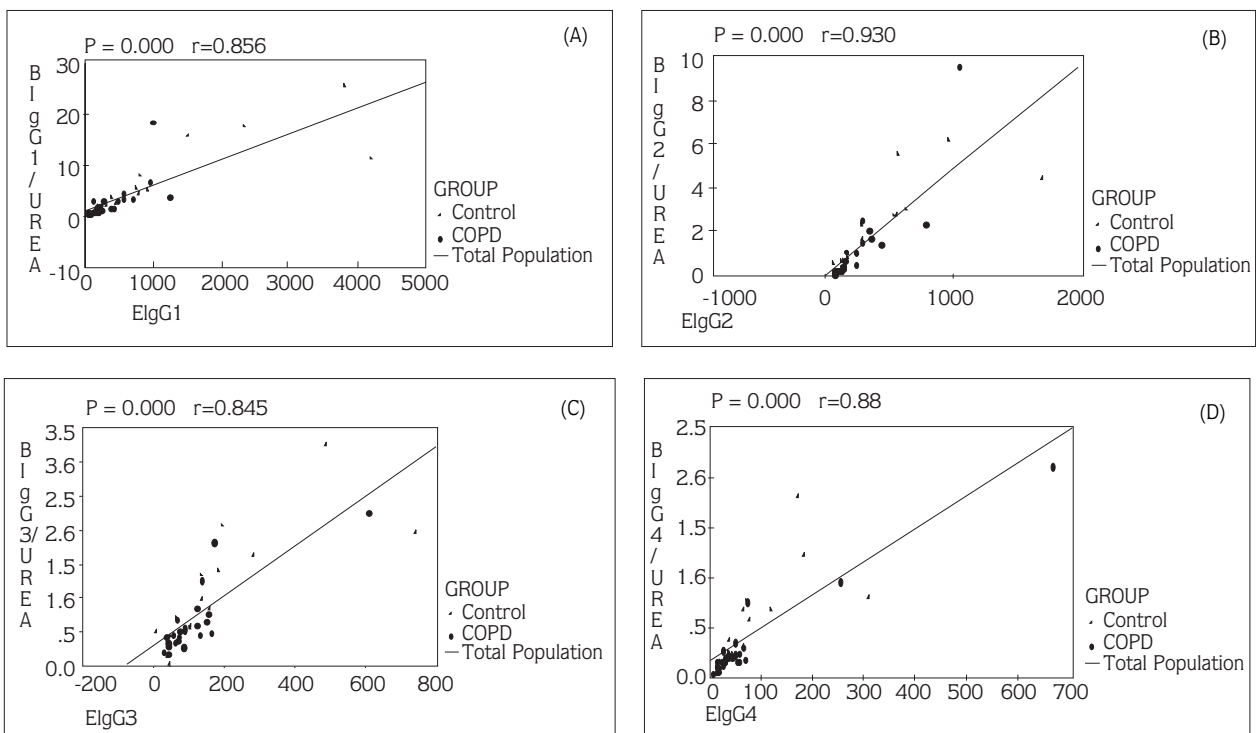
Figure 1.   Correlations between IgGsc levels to urea ratios in BAL and IgGsc levels in ELF.
a) Correlation between B-IgG1/urea and E-IgG1
b) Correlation between B-IgG2/urea and E-IgG2
c) Correlation between B-IgG3/urea and E-IgG3
d) Correlation between B-IgG4/urea and E-IgG4

(66%) had high values of at least one of the IgGscs, probably due to the frequent antigenic stimulation (25,29).

O'Keeffe and coworkers concluded that IgG2 in serum and FEV1 were well correlated (2). However, we could not establish any correlation between these parameters, probably due to the clinical stability of our COPD subjects.

Smoking may increase some IgGsc levels in serum and BAL fluid (30). This effect of smoking disappears in ex-

smokers within a year. In Group I, the high levels of s-IgGsc (IgG1,2,3,4) can not be explained by the effect of smoking as both groups were all ex-smokers for at least one year.

Alveolar surface epithelium and epithelial lining fluid are rich in chemical substances and proteins. ELF obtained by BAL can strongly represent the epithelial lining fluid. The low level of a substance in ELF may account for an impaired synthesis or transudation from the serum or an increased local consumption of that substance (24,30-32). IgG1 and IgG2 are synthesized in a negligible amount in the lung tissue. However, IgG3 and IgG4 are synthesized and expressed in the lung. Although serum levels of IgG1 and IgG2 were elevated, E-IgGsc levels were lower in Group I when compared to Group II. This may be due to the disturbed transudation in lung tissue or increased local consumption during lower respiratory tract infections in patients with COPD. We were unable to establish any correlation between the ELF and serum levels of IgGscs but B-IgGsc/urea ratios were strongly correlated with E-IgGsc representing BAL/urea correction as accurate as ELF measurements (Figure 1).

The deficiency of IgA, IgG2 and IgG4 in sera may cause chronic lung disease (13,15,29,33). We did not detect any deficiency of one or more IgGsc in sera of the subjects. However, we were unable to establish whether this deficiency in ELF of COPD patients was the cause of COPD or the result of acute exacerbations (Table 4).

The antibodies against several bacterial proteins are in IgG1 nature (3,34). Infections with capsulated bacteria like *H. influenzae* and *S. pneumoniae*, which usually cause acute exacerbation in COPD, may occur frequently in the IgG2 deficient patients (35). The local deficiency of IgG1 and IgG2 may be implicated for the local immunocompromisation that may be responsible for recurrent infections. This in turn can cause mucosal degeneration, which can be visually evaluated through FOB by using CBI, which was significantly higher in Group I.

In conclusion, deficiency in Igs and IgGscs has been implicated for the pathogenesis of COPD. In these patients, although the main class Ig levels are normal in the serum, IgGsc levels can be high. Despite the normal or high IgGsc levels in sera, there can be surprisingly decreased levels of IgG1 and IgG2 in the lung tissue (in ELF), probably due to deteriorated transudation or increased utilisation in the alveolar spaces or airways. This suggested an impaired local pulmonary immunological response in patients with COPD. Finally, we conclude that deficiency of IgG1 and IgG2 in ELF may responsible for the recurrent acute exacerbations in patients with COPD but further studies need to be done in large series about local humoral immunodeficiency in the lung.

Correspondence author:

Demet KARNAK

Ankara University Medical Faculty

Department of Chest Diseases and Tuberculosis

06100 Cebeci, Ankara - TURKEY

References

1. Ammann AJ.Antibody (B cell) immunodeficiency disorders. Basic and Clinical Immunology. (Eds Stities DP, Terr AI). Prentice-Hall International. California 1991, pp 323-340.

2. O'Keeffe S, Gzel A, Drury R, Cullina M, Greally J, Finnegan P. Immunoglobulin G subclasses and spirometry in patients with chronic obstructive pulmonary disease. Eur Respir J 4:932-936, 1991.

3. Klaustermeyer WB, Gianos ME, Kurohara ML, Dao Ht, Heiner DC. IgG subclass deficiency in obstructive lung disease. Chest 102:1137-42, 1992.

4. American Thoracic Society: Standards for the diagnosis of the diagnosis and care of patients with chronic obstructive pulmonary disease and asthma. Am Rev Respir Dis 152:77-120, 1995.

5. Cooper MD, Lawton AR. Disorders of immune system, connective tissue and joints. (Eds Wilson JD, Braunwald E, Isselbacher KJ). Harrison's Principles of Internal Medicine McGraw-Hill, Incorporation Book Company. New York 1991, pp:1395-1412.

6. Daniele RP. Immune defenses of the lungs. Pulmonary Diseases and Disorders. (Ed Fishman AP). McGraw-Hill Book Company. New York, 1988, pp:589-597.

7. Ericsson CH, Svartengren M, Mossberg B, Camner P. Bronchial reactivity, lung function, and serum immunoglobulin E in smoking discordant monozygotic twins. Am Rev Respir Dis 147(2): 296-300, 1993.

8. Melvold R. Review of immunology. Allergic Diseases, Diagnosis and Management. (Eds Patterson R, Grammer LC, Greenberger PA) JB Lippincott Company. Philadelphia 1993, pp:11-21.

9. Weir DM: Immunology an outline for students of medicine and biology in acquired immunity. Churchill Livingstone. Edinburgh London (Fifth edition) 1984, pp: 64-78.

10. Seaton A, Seaton D, Leitch AG. Lung defenses and immunology. Respiratory Diseases. (Eds Crofton and Douglas) Blackwell Scientific Publications. London 1989, pp: 95-103.

11. Goodman JW. Immunoglobulin structure and function. Basic and Clinical Immunology. (Eds Stities DP and Terr AI). Prentice-Hall International. California 1991, pp: 109-121.

12. Lanier L. Cells of the immune response: Lymphocytes & mononuclear phagocytes. Basic and Clinical Immunology. (Eds Stities DP and Terr AI). Prentice-Hall International. California 1991, pp: 61-72.

13. Schur PH. IgG subclasses-a review. Ann Allergy 58: 89-99, 1987.

14. Hammarström L, Smith ClE. The use of intravenous IgG as prophylaxis and for treatment of infections. Infection 5: 314-324, 1990.

15. Söderström T, Söderström R, Avanzini A, Brandtzaeg P, Karlsson G, Hanson LA. Immunoglobulin G subclass deficiencies. Arch Allergy Appl Immun 82: 476-480, 1987.

16. Baets F, Pauwels R, Scramme I, Leroy J. IgG subclass specific antibody response in recurrent bronchitis. Arch Dis Child 66: 1378-1382, 1991.

17. Moss RB, Camack MA, Esrig S. Deficiency of IgG4 in children: Association of isolated IgG4 deficiency with recurrent respiratory tract infection. J Pediatr 120: 16-21, 1992.

18. Thompson AB, Rennard SI: Assessment of airways inflammation utilizing bronchoalveolar lavage. Clin Chest Med 9: 635-642, 1988.

19. Rennard SI, Basset G, Lecossier D, O'Donnell KM, Pinkston P, Martin PG, Crystal RG. Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. J Appl Physiol 60: 532-538, 1986.

20. Martinek RG. Review of methods for determining urea nitrogen in biologic fluids. J Am Med Technol 31: 678, 1969.

21. Hamilton RH. Human IgG subclass measurements in the clinical laboratory. Clin Chem 33: 1707-1725, 1987.

22. Mancini G, Vaerman JP. Protides of biological fluids. XI. Colloqium. (Ed Peters H). Elsevier Publishing Co. Amsterdam 1964, pp: 370.

23 Out TA, Van De Graaf EA, Van Den Berg NJ, Jansen HM. IgG subclasses in bronchoalveolar lavage fluid from patients with asthma. Scand J Immunol 33: 719-727, 1991.

24. Baldwin Dr, Wise R, Andrews JM, Honeybourne D. Microlavage: a technique for determinating the volume of epithelial lining fluid. Thorax 46(9): 658-662, 1991.

25. Horan MA, Leahy BC, Fox RA. Immunological abnormalities in patients with chronic bronchial suppuration: a possible relationship with endotoxemia. Br J Dis Chest 78: 66-74, 1984.

26. Ward C, Duddridge M, Fenwick J, Gardiner PV, Fleetwood A, Hendrick DJ, Walters EH. Evaluation of albumin as a reference marker of dilution in bronchoalveolar lavage fluid from asthmatic and control subjects. Thorax 48: 518-522, 1993.

27. Vyve TV, Chanez P, Bernard A, Bousquet J, Godard P, Lauwerijs, Sibille Y. Protein content in bronchoalveolar lavage fluid of patients with asthma and control subjects. J Allergy Clin Immunol 95(1): 60-68, 1995.

28. Wichert PV, Joseph K, Müller B, Franck WM. Bronchoalveolar lavage quantitation of intraalveolar fluid. Am Rev Respir Dis 147: 148-152, 1993.

29. Stanley PJ, Corbo G, Cole PJ. Serum IgG subclasses in chronic and recurrent respiratory infections. Clin Exp Immunol 58: 703-708, 1984.

30. Merrill WW, Naegel GP, Olchowski JJ, Reynolds HY. Immunoglobulin G subclass proteins in serum and lavage fluid of normal subjects. Am Rev Respir Dis 131: 584-587, 1985.

31. Schur PH, Borel H, Gerfond EW, Alper CA, Rosen FS. Selective gamma G globulin deficiencies in patients with recurrent pyogenic infections. N Eng J Med 283: 631-633, 1970.

32. Settipane GA, Pudupakkam RK, Mc Gowan JH. Corticosteroid effect on immunoglobulins. J Allergy Clin Immunol 62: 162-166, 1978.

33. Gracia J, Rongo MJ, Morell F,Vendrell M, Miravitlless M, Cruz MJ, Codina R, Bofill JM. IgG subclass deficiencies associated with bronchiectasis. Am Respir Crit Care Med 153: 650-655, 1996.

34. Klaustermeyer WB, Wong SC, Schoettler JJ, Gianos ME, Heiner DC. Quantitative immunoglobulins and IgG subclasses in patients with corticosteroid dependent reversible airway obstruction. Ann Allergy 63: 327-330, 1989.

35. Siber GR, Schur H, Aisenberg AC, Weitzman SA, Schiffman G. Correlation between serum IgG2 concentrations and the antibody response to bacterial polysaccharide antigens. N Eng J Med 303: 178-182, 1980.