Eur J Med Res (2007) 12: 145-151

Vascular Endothelial Growth Factor, Lipoporotein-associated Phospholipase A2, sP-Selectin and Antiphospholipid Antibodies, Biological Markers with Prognostic Value in Pulmonary Hypertension Associated with Chronic Obstructive Pulmonary Disease and Systemic Lupus erithematosus

C. Tanaseanu[1], S. Tudor[1], I. Tamsulea[1], D. Marta[2], G. Manea[2], E. Moldoveanu[2]

[1]Internal Medicine Department, "Sf. Pantelimon" Emergency Hospital, Bucharest, Romania
[2]Ultrastructural Pathology Department, "Victor Babes" National Institute of Research and Development in Pathology and Biomedical Sciences, Bucharest, Romania

Abstract
Objective: Pulmonary arterial hypertension (PH) is a progressive disease with a poor prognosis that ultimately leads to right ventricular failure and death. The pathogenesis of severe PH seems to be related to inflammatory responses and coagulation disturbances. Many diseases can develop PH in their course, thus aggravating their outcome.

The objective was to investigate the values of vascular endothelial growth factor (VEGF), sP-selectin, lipoporotein-associated phospholipase A2 (PLA2-LDL), antiphospholipid antibodies (APLA) and their relation with PH, in systemic lupus erithematosus (SLE) and chronic obstructive pulmonary disease (COPD), two conditions in which the occurrence of PH is frequent.
Design: Prospective clinical study.
Setting: A University Department of Internal Medicine, a National Institute of Research.
Patients: 30 SLE patients [15 patients without PH (group I) and 15 patients with PH group II)], 30 patients with COPD [15 patients without PH (group III) and 15 patients with PH (group IV)] and 10 healthy controls, selected by clinical, immunological, echocardiographycal criteria and pulmonary functional tests.

Main outcome measures: VEGF, sP-selectin and PLA2-LDL level in plasma and presence of antiphospholipids antibodies (lupus anticoagulant, anticardiolipin and anti ß2 GPI) in plasma.
Results: In patients with PH, the values of VEGF were significantly increased [group II (1023.1) and IV (904.3)] compared with group I (744.2), III (356.4), and controls (330.3). The values of sP-selectin in group II (9.7), and IV (10.4) were also increased compared with controls (6). APLA were present in all patients in group II (100%), and in 8 patients in group IV (53%), while in the other groups the frequency was low (33% group I and 13% group III). PLA2-LDL activity was higher in group II (429.1) and group IV (394.5) than in group I (317.8), group III (343.2) and controls (256.3).
Conclusion: PH is a severe complication in COPD and SLE. The increased values of VEGF, PLA2-LDL and P-selectin in patients with long standing PH are related to severe endothelial dysfunction and may have prognostic values. APLA may have pathogenic value in SLE patients with PH. APLA are possibly implicated in the pathogenesis of PH in these diseases. VEGF, APLA and sP-selectin may constitute new therapeutic targets for PH.

Key words: pulmonary arterial hypertension, systemic lupus erithematosus, chronic pulmonary obstructive disease, vascular endothelial growth factor, sP-selectin, lipoporotein-associated phospholipase A2, antiphospholipid antibodies.

Introduction

Pulmonary arterial hypertension (PH) is a disease of the small pulmonary arteries, characterized by vascular narrowing leading to a progressive increase in pulmonary vascular resistance. The consequence of this increased right ventricular overload is the failure of the afterload intolerant right ventricule.

PH has a multifactorial pathobiology, but pulmonary vascular proliferation and remodeling is a common feature for all forms of PH. Subsequent intimal thickening, fibrosis and *in situ* thrombosis lead to plexiform lesions [1, 2].

The stimuli responsible for these processes involve transmural pressure, growth factors, inflammatory cytokines and coagulation disturbances [1]. According to the Venice classification PH is associated with many diseases [2]. Among them chronic obstructive pulmonary disease (COPD) and systemic lupus erythematous (SLE), two diseases quite common in the middle aged population, develop in their course PH, a condition related with poor outcome.

We try to investigate the values of some biological markers as predictors of PH, markers that were found to be highly expressed in inflammatory processes of the arterial wall, in patients with SLE and COPD, diseases in which the occurrence of PH is frequent

[3, 4].

Plasma platelet-activating factor acetylhydrolase also known as lipoprotein-associated phospholipase A2 (PLA2-LDL) induces the release of inflammatory mediators in different inflammatory conditions [5, 6, 7].

Soluble P-selectin (sP-selectin) is considered to be a marker of platelet activation. sP-selectin is present in dense granules of platelets and endothelial cells (Weibel-Palade bodies). It is expressed only on activated cells, in response to different stimuli: hypoxia, reactive oxygen species, cytokines and thrombin [8].

Vascular endothelial growth factor (VEGF) is a multifunctional peptide capable of inducing receptor –mediated endothelial cell proliferation and angiogenesis, promoting collateral vessels formation in ischemic cardiac muscle. VEGF is present in pulmonary and cardiac vasculature but may also arise from platelets [1].

Antiphospholipids antibodies (APLA) are frequently present in SLE and also seem to be connected to many infectious agents, inducing endothelial dysfunction and a hypercoagulable state [9, 10, 11].

Materials and Methods

The study lot consisted in 30 patients with SLE [15 patients without PH (group I), 15 patients with PH (group II)], 30 COPD patients [15 patients without PH (group III) and 15 patients with PH (group IV)] and 10 healthy controls. Patients were selected by clinical and immunological criteria, ultrasonography and pulmonary functional tests.

The normal controls were healthy, life-long non-smoking volunteers who had no history of lung or autoimmune diseases. All patients with COPD were enrolled using the data base of the respiratory patients of Emergency Clinical Hospital "St. Pantelimon". They had a history of former smoking (>20 pack-yrs) and pre-bronhodilatator forced expiratory volume in one second (FEV1) ≤ 80 % predicted, FEV1/FVC < 70% predicted, and previous exacerbations.

COPD patients medication consisted in theophylline and in some cases in an inhaled anticholinergic drug, oral or inhaled corticosteroids, anticoagulant treatment, oxygen and statins [12, 13, 14, 15]. No patients received medication during the 12-h period preceeding the spirometric study.

SLE patients received corticosteroids, cyclophosphamide, hydroxycloroquine, and endothelial protection treatment (aspirin, statins, ACE-inhibitors ± anticoagulants) [16, 17, 18].

All patients with COPD and SLE were clinically stable, and none had a history of respiratory infection for at least the 4-week period preceding the study.

Spirometry was performed using a Yaeger spirometer. Chronic airflow obstruction was defined as an FEV1/vital capacity ratio < 70% and an FEV_1 ≤ 80% of predicted values [19].

The diagnosis of COPD should be considered in any patient who has the following:

- symptoms of cough
- sputum production or
- dyspnoea or
- history of exposure to risk factors for the disease.

The diagnosis requires spirometry; post-bronchodilator FEV1/FVC < 70% confirms the presence of airflow limitation that is not fully reversible.

The carbon monoxide diffusing capacity of the lung (DLCO) was measured only in some SLE patients.

Patients underwent routine laboratory tests, bacteriological and serological tests, immunologic determination for SLE diagnosis (-antiDNAds, -antiRo, - antiSm antibodies and components of complement system).

Patients with COPD were evaluated according to standard GOLD criteria (clinical assessment, ventilatory functional tests and oximetry) [20].

All patients were radiologicaly evaluated to exclude active pulmonary inflammatory processes and/or malignancies.

ECG and echocardiography were performed for diagnostic to the criteria of PH and in order to differentiate between acute and chronic thromboembolic disease [21, 22, 23, 14].

PH is defined as a mean resting pulmonary artery pressure >25mmHg or a mean pulmonary artery pressure >30mmHg with exercise. The World Health Organization definition is a pulmonary artery systolic pressure >40mmHg allowing for echographic estimates [2, 14].

The results of bacteriological and serological tests in COPD patients with previous hospitalization for respiratory tract infections were obtained from hospital files.

Patients were followed-up for a mean period of 12

Classification of Severity of COPD

GOLD stage	Characteristics
0: At risk	Normal spirometry Chronic symptoms (cough, sputum production)
I: Mild COPD	FEV1/FVC < 70% FEV_1 ≥ 80% predicted (with or without chronic symptoms cough, sputum production)
II: Moderate COPD	FEV1/FVC < 70% 50% ≤ FEV_1< 80% predicted (with or without chronic symptoms: cough, sputum production)
III: Severe COPD	FEV_1< 70% 30% ≤ FEV1/FVC <50% predicted (with or without chronic symptoms: cough, sputum production)
IV: Very severe COPD	FEV_1 /FVC < 70% FEV_1 <30% predicted or FEV_1 < 50% predicted plus chronic respiratory failure

*FVC forced vital capacity; FEV_1 forced expiratory volume in one second;
Respiratory failure: arterial partial pressure of oxygen (PaO2) less than 60mmHg (8.0kPa) and or without arterial partial pressure of CO2 (PaCO2) higher than 50mmHg (6,7kPa), while breathing air at sea level.

months, with two sequentially determinations of clinical, echographic and biological parameters.

Biochemical Assay

All plasma samples were frozen and stored at -80 °C until analysis.

PLA2-LDL activity was measured using the spectrophotometric method described by Kosaka et al. using the Azwell Auto PLA2-LDL kit [24]. Enzymatic activity was measured by the change in absorbance difference between 405 and 505 nm, and it is expressed as IU/L.

VEGF and sP-selectin were measured with commercial ELISA kit (R&D Systems, Germany) and the results were expressed as pg/ml and ng/ml.

Determination of antiphospholipid antibodies (APLA) included: lupus anticoagulant – coagulation test LAC screen and test LAC confirm (+ >1.5); anticardiolipin (+ >23GPLu, >11MPLu) and anti-β2 GPI antibodies (+ >20ui/ml) with commercial ELISA kit (BIORAD, USA); C_3 and C_4 with nephelometric method using Proton (UK) reagent.

Statistical Analysis

All values are presented as mean ± standard deviation (SD). The statistical analysis was performed with the SPSS statistical package. The significance of correlations was evaluated by determining Spearman's rank correlation coefficients. A two-tailed p-value < 0.05 was considered significant.

Results

Basic characteristics of the 30 COPD patients and 30 SLE patients are shown in (Table1).

APLAs were present in group IV patients, mainly in those patients with recent infections (from 12 COPD patients with APLA, 8 patients had PH and previous respiratory infection with Chlamydia pneumoniae, Streptococcus pneumoniae and Haemophylus influenzae). All SLE patients with PH were positive for APLA.

Table 1. Basic characteristics of patients.

variable	SLE (gr. I)	SLE (gr. II)	COPD (gr. III)	COPD (gr. IV)
mean age	35 ± 15	45 ± 10	60 ± 15	67 ± 12
gender F/M	12/3	11/4	4/11	7/8
smoking	2 (13%)	3 (20%)	15(100%)	12 (80%)
HTA*	3(20%)	8(53%)	4(27%)	5 (33%)
HF*	0	2 (13%)	0	8 (53%)
FEV1* < 60%	0	1 (6,6%)	5 (33%)	9 (60%)
DLco <75%	0	4 (26,6%)	ND	ND
SaO2 <90%	0	3 (20%)	5 (33%)	9 (60%)
APLA	5 (33%)	15 (100%)	2 (13%)	8 (53%)

*arterial hypertension-HTA, heart failure-HF, FEV1-forced expiratory volume in 1 sec.

Table 2. Clinical and laboratory characteristics and treatment of patients with SLE.

Characteristics	Gr.I	GrII
SLEDAI score	3,9 ± 1.9	4,2 ± 1.6
Raynaud's phenomenon (%)	3 (20%)	4 (26%)
Articular involvement	11 (73%)	9 (60%)
Cardiac involvement	4 (26%)	7 (46%)
Cerebrovascular disease (%)	3 (20%)	2 (13%)
Pulmonary involvement (other that PH)	4 (26%)	3 (20%)
Renal involvement	3 (26%)	3 (20%)
C_4 (mg/l)	0,141 ± 0.07	0,125 ± 0.015
Anti-DNAds (%)	9	10
Anti-Sm (%)	2	0
Anti-Ro (%)	6	7
Anti-cardiolipin (%)	3 (20%)	7 (46%)
Anti b2 GPI (%)	1 (6 %)	2 (13%)
Lupus anticoagulant (%)	4 (26%)	11 (73%)
Treatment		
Prednisone	100	100
Cyclophosphamide (%)	13	26
Hydroxychloroquine (%)	100	100
Aspirin (%)	100	100

Clinical and laboratory characteristics and treatment of patients with SLE are presented in Table 2.

Gold stages of COPD patients are presented in Table 3.

VEGF levels were increased in patients with PH (group II and group IV) especially in COPD patients, compared to patients without PH (group I and group III) and controls (Table 4).

During the follow up period, patients with stable or progressive PH, worsening HF, and progressive severe COPD displayed increased values of VEGF after sequential determination (Table 4, Fig 1). Similar results were obtained in SLE patients with long-standing PH (Table 4, Fig 2).

Compared with controls, COPD and SLE patients, but mainly SLE patients with PH, showed an increase in the level of PLA2-LDL (Table 4).

Elevated serum sP-selectin levels were observed in SLE and COPD patients with PH (group II and group IV), compared to controls and to patients without PH (group I and group III) (Table 4). A significant correlation was observed between VEGF and sP-selectin in COPD patients with long standing PH (Fig.3).

Evolution:

In SLE patients, from group I, 12 patients remained stable and 3 had active disease with flare up episodes. In group II, 9 patients remained stable, 5 had flare up episodes (3 with increased pulmonary artery pressure

Table 3. GOLD stages of COPD patients.

COPD stages	Gr.III	Gr.IV
0 :at risk	-	-
I : mild	7	4
II : moderate	3	2
III : severe	4	6
IV : very severe	1	3

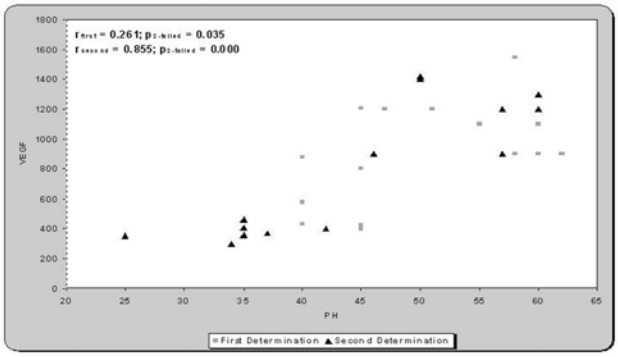

Fig 1. The relationship between VEGF activity and PH (group IV).

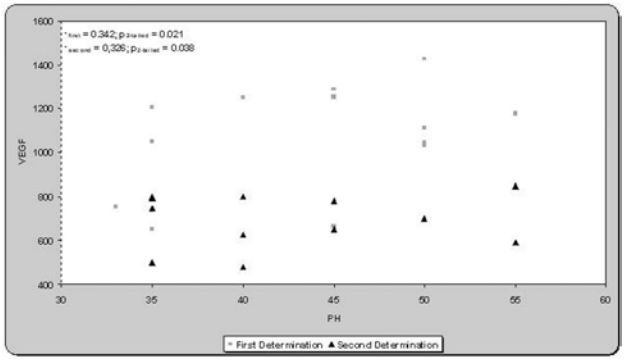

Fig 2. The relationship between VEGF activity and PH (group II).

Table 4. Mean value and standard deviation for VEGF, PLA2-DL d sP-selectin.

group	parameter	Mean value ± SD	
		First determination	Second determination
Control	VEGF	330.3 ± 84.16	
	PLA2-LDL	256.3 ± 65.11	
	sP-selectin	6 ± 2	
Group I	VEGF	744.2 ± 425.1	450.2 ± 104.06
	PLA2-LDL	317.8 ± 109.04	345.5 ± 9.12
	sP-selectin	7.9 ± 3.9	7 ± 3.2
Group II	VEGF	1023.1 ± 259.07	680.4 ± 134.04
	PLA2-LDL	429.1 ± 111.01	345.6 ± 92.6
	sP-selectin	9.7 ± 5.9	7.8 ± 3.4
Group III	VEGF	356.4 ± 94.51	380.9 ± 112.40
	PLA2-LDL	343.2 ± 94.51	369.8 ± 125.4
	sP-selectin	7.8 ± 3.6	7 ± 2.3
Group IV	VEGF	904.3 ± 423.6	791.5 ± 431
	PLA2-LDL	394.5 ± 108.48	352 ± 96.23
	sP-selectin	10.4 ± 3.9	9.5 ± 3.2

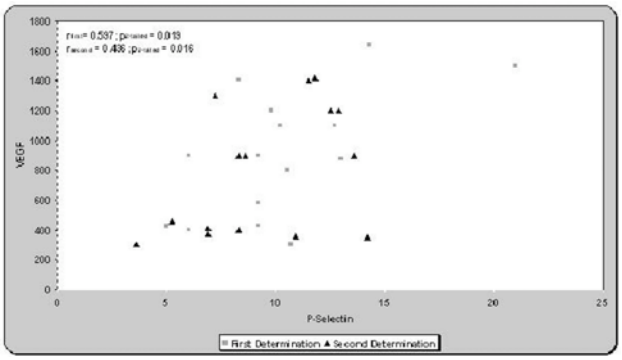

Fig 3. The relationship between VEGF activity and sP-selectin (group IV).

compared to basal values) and 1 patient had an acute coronary syndrome (unstable angina).

In COPD patients, from group III, 10 patients remained stable, 3 patients improved their clinical state and pulmonary functional tests and 2 aggravated their clinical symptoms and displayed decreased pulmonary functional tests.

In group IV, 6 patients were stable, 5 patients had episodes of worsening HF, and 4 patients developed transient acute respiratory insufficiency (*in situ* thrombosis) with transient increase of pulmonary artery pression.

DISCUSSION

The pulmonary circulation is a low–pressure, high-flow system with a great capacity for recruitment of normally unperfused vessels. As a consequence, the walls of pulmonary arteries are thin, in keeping with their low transmural pressure [1]. Regardless of the etiology, PH appears as an extremely serious condition that is difficult to identify early owing to the insidious nature of early–stage symptoms.

Endothelial dysfunction is a key event of PH pathophysiology, leading to complex vascular disturbances. Endothelial cells express markers of angiogenesis, such as VEGF and its receptors [1, 2, 25].

The results of this study show an increased level of VEGF in serum of patients with moderate and severe COPD associated with long standing PH, and normal levels in COPD patients without PH. COPD is associated with structural and functional changes in the pulmonary circulation that commence at an early stage [3, 26, 28]. VEGF is mainly implicated in the maintenance of vascular endothelial cell function and in vascular cell proliferation. Some of these functions are performed through NO-dependent mechanisms [29]. It was hypothesized that in the early stages of COPD, VEGF might have contributed to the structural remodeling of pulmonary arteries, presumably by enhancing the proliferation and intimal migration of SMCs [26, 27, 28, 30]. In COPD patients the macrophage VEGF transcription may be stimulated by ROS generated by activated neutrophiles as a possible consequence of frequent exacerbations [30, 31].

Recent studies have shown increased expression of VEGF and its receptors in endothelial cells and in intimal and medial vascular smooth muscle in COPD patients. An increased VEGF level was also observed in serum and in induced sputum of COPD patients [32]. In severe COPD, a loss of lung tissue may be correlated with a low concentration of VEGF in plasma and in induced sputum [27, 29, 33].

VEGF and its receptors may be associated with epithelial cell viability during airway wall remodeling [32, 33]. However, further studies are needed to clarify the role of VEGF at different stages of COPD with and without PH.

In SLE patients the levels of VEGF were increased in both groups of patients, reflecting diffuse and continuous endothelial damage and possibly some participation in the inflammatory process [9, 25]. Endothelial dysfunction and impaired NO synthesis, which are present in smokers and in patients with mild COPD and SLE may facilitate the up-regulation of VEGF in pulmonary vessels [29, 32]. The degree of lymphocytic infiltrate is correlated with the extent of intimal thickening [32]. However it is unknown, to what extent these recruited inflammatory cells are a cause or a consequence of vascular remodeling. Conceivably, inflammatory stimuli related to flare up episodes in SLE or to exacerbation of COPD could be potential triggers for VEGF up-regulation at initial stages of the process. Many inflammatory mediators have been shown to induce VEGF mRNA and protein expression [32]. Inflammatory cells (macrophages, neutrophiles, lymphocytes) contribute to endothelial lesions by increasing oxidative stress and prothrombotic environment – aggravating hypoxia. Oxidative stress leads to free radical attack on DNA, lipids, and proteins [33]. In both diseases, PLA2-LDL is increased compared to controls, but especially in SLE patients with PH, possibly reflecting more endothelial damage. Increased levels of PLA2-LDL may be an evidence of increased production of platelets activating factor (PAF), and also an increased oxidative stress, representing a link between inflammation and hypoxia [5, 6].

Thrombotic lesions and platelets dysfunction are potentially important processes in PH. Increased levels of sP-Selectin were observed in SLE and COPD patients with PH, reflecting pulmonary endothelial damage and platelet activation [34, 35]. The results of our study showed a significant relationship between VEGF and sP-selectin in patients with COPD and long-standing PH. This fact may be related to persistent platelet activation [36]. The presence of high levels of VEGF and PAF (reflected by PLA2-LDL) in patients with PH could increase the inflammatory processes by exacerbating the adhesion of neutrophiles to activated endothelial cells [37]. In PH, platelets present dysfunctions, and release various procoagulant factors. Moreover, there are described enhanced interactions between platelets and vessels wall, contributing to microvascular damage. Selectin –mediated adhesion of leukocytes to the vascular endothelium is a key early event in the initiation of the inflammatory response. However, *in situ* thrombosis and platelet dysfunction may be at the same time the cause and the consequence of PH.

APLA are involved in pathogenesis of PH in pa-

tients with SLE, reflecting severe endothelial dysfunction and long standing inflammation [43, 44, 45]. In COPD patients, APLA were present mainly in those patients with previous infections. It is well known that infection may be a trigger for the induction of pathogenic APLA, associated with severe clinical thrombotic events [10, 11]. These autoantibodies induced by frequent exacerbation of COPD may play an important role in enhancing endothelial damage leading to PH. APLA and selectin may be targets for therapeutic interventions in patients with PH [40, 41, 42]. The outcome of patients with PH is better in SLE, than in COPD, possibly due to the reversibility of the inflammatory processes as a result of specific immunomodulatory associated treatment [17, 18, 46].

CONCLUSIONS

PH is a common and severe complication in SLE and COPD. In both diseases patients with PH showed increased values of VEGF, PLA2-LDL and sP-selectin, supporting the evidence that diffuse endothelial damage and platelet activation is associated with long standing PH, more severe disease and poorer outcome. PLA2-LDL, reflecting PAF levels and increased oxidative stress, is considered to be a link between hypoxia, inflammation and endothelial dysfunction. APLA may be implicated in the pathogenesis and progression of PH in both diseases. The assay of these biomarkers may have important prognostic value and some of them are possible therapeutic targets.

Acknowledgements: The presented study was supported by the National Research Program Viasan, under the patronage of the Romanian Academy of Medical Science, Viasan Grants 352/2005, 356/2005 and CEEX Grants 39/2005, 75/2006.
Footnote: Conflict of interest: The authors have declared that no conflict of interest exists.

REFERENCES

1. Humbert M, Morrell NW, Archer SL, Stenmark KR, MacLean MR, Lang IM, Christman BW, Weir EK, Eickelberg O, Voelkel NF, Rabinovitch M. Cellular and molecular pathobiology of pulmonary arterial hypertension. J Am Coll Cardiol. 2005 Jun; 43: 13S-24S.
2. Simonneau G, Galiè N, Rubin LJ, Langleben D, Seeger W, Domenighetti G, Gibbs S, Lebrec D, Speich R, Beghetti M, Rich S, Fishman A. Clinical classification of pulmonary hypertension. J Am Coll Cardiol. 2004 Jun; 43: 5S-12S. Review.
3. Voelkel NF, Cool CD, Pulmonary vascular involvement in chronic obstructive pulmonary disease. Eur Respir J Suppl. 2003 Nov; 46:28s-32s.
4. Hass C. Pulmonary hypertension associated with systemic lupus erythematosus, Bull Acad Natl Med. 2004;188(6):985-97
5. Ninio E, Phospholipid mediators in the vessel wall: involvement in atherosclerosis. Curr Opin Clin Nutr Metab Care. 2005 Mar; 8(2):123-31. Review.
6. Tanaseanu C, Moldoveanu E, Kosaka T, Tanaseanu S, Neagu M, Popescu LM. The significance of human platelet-activating factor-acetylhydrolase in patients with chronic stable angina. Eur J Intern Med. 2004 Aug; 15(5):291-297.
7. Moldoveanu E, Tanaseanu C, Tanaseanu S, Kosaka T, Manea G, Marta DS, Popescu LM. Plasma markers of endothelial dysfunction in type 2 diabetics. Eur J Intern Med. 2006 Jan; 17(1):38-42.
8. Ferroni P, Basili S, Martini F, Vieri M, Labbadia G, Cordova C, Alessandri C, Gazzaniga PP. Soluble P-selectin as a marker of platelet hyperactivity in patients with chronic obstructive pulmonary disease. J Investig Med. 2000 Jan; 48(1): 21-7.
9. Joseph JE, Harrison P, Mackie IJ, Isenberg DA, Machin SJ. Increased circulating platelet-leucocyte complexes and platelet activation in patients with antiphospholipid syndrome, systemic lupus erythematosus and rheumatoid arthritis.Br J Haematol. 2001 Nov; 115(2):451-9.
10. Blank M, Shoenfeld Y. Beta-2-glycoprotein-I, infections, antiphospholipid syndrome and therapeutic considerations. Clin Immunol. 2004 Aug; 112(2):190-9. Review.
11. Blank M, Asherson RA, Cervera R, Shoenfeld Y. Antiphospholipid syndrome infectious origin. J Clin Immunol. 2004 Jan; 24(1):12-23. Review.
12. Nishimura T, Vaszar LT, Faul JI, Zhao G, Berry GJ, Shi L, Qin D, Benson G, Pearl RG, Kao PN. Simvastatin rescues rats from fatal pulmonary hypertension by inducing apoptosis of neointimal smooth muscle cells. Circulation. 2003 Sep 30; 108(13):1640-5.
13. British cardiac society Guidelines and medical practice Comitte, and approved by the British Thoracic Society and the British society of Rheumatology Recommendations on management of pulmonary hypertension in clinical practice Heart 2001; 86(suppl 1): 1-14.
14. Galie N, Torbicki A, Barst R, Dartevelle P, Haworth S, Higenbottam T, Olschewsky H, Peacock A, Pietra G, Rubin LJ, Simonneau G, Priori SG, Garcia MA, Blanc JJ, Budaj A, Cowie M, Dean V, Deckers J, Burgos EF, Lekakis J, Lindahl B, Mazzotta G, McGregor K, Morais J, Oto A, Smiseth OA, Barbera JA, Gibbs S, Hoeper M, Humbert M, Naeije R, Pepke-Zaba J, Task Force. Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The Task Force on Diagnosis and Treatment of Pulmonary Arterial Hypertension of the European Society of Cardiology. Eur Heart J. 2004 Dec; 25(24):2243-78.
15. Agusti AGN. COPD a multicomponent disease: implications for management. Respir. Med. 2005 June; 99(6): 670-682.
16. Graninger W. Therapy of pulmonary involvement of rheumatic diseases.
Z Rheumatol. 2003 Feb; 62(1):10-5. Review.
17. Gonzalez-Lopez L, Cardona-Munoz EG, Celis A, Garcia-de la Torre I, Orozco-Barocio G, Salazar-Paramo M, Garcia-Gonzalez C, Garcia-Gonzalez A, Sanchez-Ortiz A, Trujillo-Hernandez B, Gamez-Nava JI. Therapy with intermittent pulse cyclophosphamide for pulmonary hypertension associated with systemic lupus erythematosus. Lupus. 2004; 13(2):105-12.
18. Lopes AA. Pathophysiological basis for anticoagulant and antithrombotic therapy in pulmonary hypertension. Cardiovasc Hematol Agents Med Chem. 2006 Jan; 4(1):53-9.
19. Reilly JJ, Silverman EK, Shapiro SD. Chronic obstructive pulmonary disease, 1547-1554 in Harrison's Principles of Internal Medicine 16th. Edition 2005. Editors: Kasper DL, Lony PL, Fonci AS, Braunwold E, Hansen SL, Jameson JL. The McGraro- Hill Companies Medical Publishing Division.
20. GOLD – Global initiative for chronic obstructive lung disease: global strategy for diagnosis management and prevention of COPD. Amer Respir Crit Care Med. 2001; 163:12564-76.
21. Barst RJ, McGoon M, Torbicki A, Sitbon O, Krowka MJ, Olschewski H, Gaine S. Diagnosis and differential assessment of pulmonary arterial hypertension. J Am Coll Cardiol. 2004 Jun 16; 43(12 Suppl S):40S-47S. Review.

22. Mahmud E, Raisinghani A, Hassankhani A, Sadeghi HM, Strachan GM , Auger W, DeMaria AN, Blanchard DG. Correlation of left ventricular diastolic filling characteristics with right ventricular overload and pulmonary artery pressure in chronic thromboembolic pulmonary hypertension. J Am Coll Cardiol. 2002 Jul 17; 40(2):318-24.
23. Torbicki A, Kurzyna M, Ciurzynski M, Pruszczyk P, Pacho R, Kuch-Wocial A, Szulc M. Proximal pulmonary emboli modify right ventricular ejection pattern. Eur Respir J. 1999 Mar; 13(3):616-21.
24. Kosaka T, Yamaguchi M, Soda Y, Kishimoto T, Tago A, Toyosato M, Mizuno K. Spectrophotometric assay for serum platelet-activating factor acetylhydrolase activity. Clin Chim Acta. 2000 Jun; 296(1-2):151-61.
25. Meroni PL, Borghi MO, Raschi E, Ventura D, Sarzi Puttini PC, Atzeni F, Lonati L, Parati G, Tincani A, Mari D, Tedesco F. Inflammatory response and the endothelium. Thromb Res. 2004; 114(5-6):329-34. Review.
26. Sakao S, Tatsumi K, Hashimoto T, Igari H, Shino Y, Shirasawa H, Kuriyama T. Vascular endothelial growth factor and the risk of smoking-related COPD. Chest. 2003 Jul; 124(1):323-7.
27. Tuder RM, Zhen L, Cho CY, Taraseviciene-Stewart L, Kasahara Y, Salvemini D, Voelkel NF, Flores SC. Oxidative stress and apoptosis interact and cause emphysema due to vascular endothelial growth factor receptor blockade. Am J Respir Cell Mol Biol. 2003 Jul; 29(1):88-97.
28. Santos S, Peinado VI, Ramírez J, Morales-Blanhir J, Bastos R, Roca J, Rodriguez-Roisin R, Barberà JA. Enhanced expression of vascular endothelial growth factor in pulmonary arteries of smokers and patients with moderate chronic obstructive pulmonary disease. Am J Respir Crit Care Med. 2003 May 1; 167(9):1250-6.
29. Bigatello LM. Nitric oxide: modulation of the pulmonary circulation. Minerva anestesiol. 2000 May; 66(5):307-13.
30. Dorfmuller P, Perros F, Balabanian K, Humbert M. Inflammation in pulmonary arterial hypertension. Eur Respir J. 2003 Aug; 22(2):358-63. Review.
31. Rose F, Hattar K, Gakisch S, Grimminger F, Olschewski H, Seeger W, Tschuschner A, Schermnly RT, Weissman N, Hanze j, Sibelius U, Ghofrani HA. Increased neutrophil mediator release in patients with pulmonary hypertension--suppression by inhaled iloprost. Thromb Haemost. 2003 Dec; 90(6):1141-9.
32. Kranenburg AR, de Boer WI, Alagappan VK, Sterk PJ, Sharma HS. Enhanced bronchial expression of vascular endothelial growth factor and receptors (Flk-1 and Flt-1) in patients with chronic obstructive pulmonary disease. Thorax. 2005 Feb; 60(2):106-13.
33. Kanazawa H, Yoshikawa J. Elevated oxidative stress and reciprocal reduction of vascular endothelial growth factor levels with severity of COPD.
Chest. 2005 Nov; 128(5):3191-7.
34. Sakamaki F, Kyotani S, Nagaya N, Sato N, Oya H, Satoh T, Nakanishi N. Increased plasma P-selectin and decreased thrombomodulin in pulmonary arterial hypertension were improved by continuous prostacyclin therapy. Circulation. 2001 Feb 6; 103(5):779.
35. Lopess AA. Pathophysiological basis for anticoagulant and antithrombotic therapy in pulmonary hypertension. Cardiovasc Hematol Agents Med Chem. 2006 Jan; 4(1):53-9.
36. Martini F, Riondino S, Basili S, Bertazzoni G, Ferroni P. In vivo platelet activation is associated with increased plasma levels of vascular endothelial growth factor. J Thromb Haemost. 2003 Apr; 1(4):853-4.
37. Rollin S, Lemieux C, Maliba R, Favier J, Villeneuve LR, Allen BG, Soker S, Bazan NG, Merhi Y, Sirois MG. VEGF-mediated endothelial P-selectin translocation: role of VEGF receptors and endogenous PAF synthesis. Blood. 2004 May 15; 103(10):3789-97. Epub 2004 Feb 5.
38. Pereira J, Alfaro G, Goycoolea M, Quiroga T, Ocqueteau M, Massardo L, Perez C, Saez C, Panes O, Matus V, Mezzano D. Circulating platelet-derived microparticles in systemic lupus erythematosus. Association with increased thrombin generation and procoagulant state. Thromb Haemost. 2006 Jan; 95(1):94-9.
39. Nagahama M, Nomura S, Ozaki Y, Yoshimura C, Kagawa H, Fukuhara S. Platelet activation markers and soluble adhesion molecules in patients with systemic lupus erythematosus. Autoimmunity. 2001; 33(2):85-94.
40. Romano SJ. Selectin antagonists: therapeutic potential in asthma and COPD. Treat Respir Med. 2005; 4(2):85-94. Review.
41. Schumacher A, Liebers U, John M, Gerl V, Meyer M, Witt C, Wolff G. P-selectin glycoprotein ligand-1 (PSGL-1) is up-regulated on leucocytes from patients with chronic obstructive pulmonary disease. Clin Exp Immunol. 2005 Nov; 142(2):370-6.
42. Ekdahl KN, Bengtsson AA, Andersson J, Elgue G, Ronnblom L, Sturfelt G, Nilsson B. Thrombotic disease in systemic lupus erythematosus is associated with a maintained systemic platelet activation. Br J Haematol. 2004 Apr; 125(1):74-8.
43. Joseph JE, Harrison P, Mackie IJ, Isenberg DA, Machin SJ. Increased circulating platelet-leucocyte complexes and platelet activation in patients with antiphospholipid syndrome, systemic lupus erythematosus and rheumatoid arthritis. Br J Haematol. 2001 Nov; 115(2):451-9.
44. Pierangeli SS, Espinola RG, Liu X, Harris EN. Thrombogenic effects of antiphospholipid antibodies are mediated by intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1, and P-selectin. Circ Res. 2001 Feb 2; 88(2):245-50.
45. Pierangeli SS, Harris EN. Probing antiphospholipid-mediated thrombosis: the interplay between anticardiolipin antibodies and endothelial cells. Lupus. 2003; 12(7):539-45. Review.
46. Godeau B, Piette JC. The significance and treatment of antiphospholipid antibodies. Presse Med. 2004 Aug 28; 33(14 Pt 1):944-52. Review.

Received: June 5, 2006 / Accepted: December 20, 2006

Address for correspondence:
Elena Moldoveanu, Ph.D.
National Institute of Research and Development
in Pathology and Biomedical Sciences "Victor Babes"
Ultrastructural Pathology Department
99-101, Splaiul Independentei
050096, Bucharest