The Pharma Innovation Journal 2014; 3(9): 27-31

www.ThePharmaJournal.com

The Pharma Innovation

ISSN: 2277- 7695
TPI 2014; 3(9): 27-31

www.thepharmajournal.com
Received: 09-09-2014
Accepted: 15-10-2014

Pertseva T. O.
State Establishment "Dnipropetrovsk Medical Academy of Health Ministry of Ukraine", Department of Therapy and Endocrinology, Ukraine, Dniepropetrovsk, 49000, 13, Batumskaya str.

Kireyeva T.V.
[1]State Establishment "Dnipropetrovsk Medical Academy of Health Ministry of Ukraine", Department of Therapy and Endocrinology, Ukraine, Dniepropetrovsk, 49000, 13, Batumskaya str.

Shtepa O.O.
State Establishment "Dnipropetrovsk Medical Academy of Health Ministry of Ukraine", Department of Therapy and Endocrinology, Ukraine, Dniepropetrovsk, 49000, 13, Batumskaya str.

Correspondence:
Shtepa O.O.
State Establishment "Dnipropetrovsk Medical Academy of Health Ministry of Ukraine", Department of Therapy and Endocrinology, Ukraine, Dniepropetrovsk, 49000, 13, Batumskaya str.

Surfactant Protein D And C-Reactive Protein in patients with lower respiratory tract infections

Pertseva T. O., Kireyeva T.V., Shtepa O.O.

Abstract
In the world researches the role of destabilization of surfactant system of the lungs is being studied more and more often, as well the role of proteins being the part of it, and which play an important role in providing its stability. Considering that, the aim of our work was assessing diagnostic significance of surfactant protein D (SP-D) in patients with community acquired pneumonia (CAP) and infectious exacerbation of chronic obstructive pulmonary disease (IE COPD) as SP-D provides development of adequate immune response in the action of components of the outer environment and pathogens, being a marker of pathogenic microorganisms, destined to be destroyed by the immune system and being attractant for the immune cells, i. e. increases phagocytosis efficacy.
Percentage ratio of infectious agents revealed in patients with CAP and IE COPD made up 37% and 45% correspondingly. Index of SP-D levels in patients of both groups with identification of causative agent and without it did not reliable difference (p=0,658; p=0,835). Plasma level of SP-D in patients with CAP and IE COPD was reliably higher as compared with control group (p=0,002; p< 0,001). However, reliable difference between findings of patients with CAP and IE COPD was not revealed (p< 0,144). Plasma level of C-RP was reliably higher in patients of both groups as compared with control group (p< 0,001). C-RP level was reliably higher in patients with CAP relatively patients with IE COPD (p< 0,001). Correlation link between C-RP and SP-D was not revealed, this is probably connected with various specificity of theses markers for infectious processes of the respiratory system.
To our opinion, in diagnostic practice it is expedient to use SP-D and C-RP markers together to more fuller characteristics of the inflammatory process, both as systemic inflammatory response, and as characteristics of the degree of pulmonary tissue lesion, developing as a result of destabilization of lung surfactant system.

Keywords: community acquired pneumonia, infectious exacerbation of chronic obstructive pulmonary disease, surfactant protein D, C-reactive protein, infectious agents of lower respiratory ways, inflammatory process of lower respiratory ways.

1. Introduction

Lower respiratory tract infections (LRTI) which occupy one of the leading places in the structure of morbidity and mortality in the world remain the most prevalent acute pathologies of a human being; they become the cause of temporary loss of ability to work and may become the cause of patients' disability [17, 19, 20]. Role of destabilization of lung surfactant system (LSS) in the pathogenesis of pulmonary tissue lesion which may impact or cause development of some or other disease is being studied more and more often in the world's scientific works. [17, 18].

As it is known, phosphlipides, composing approximately 90% and specific proteins of surfactant, composing approximately 10% play a leading role in the maintaining structural organization of LSS, process of lung remodeling, defense of macroorganism from intervention of infection agents are the main chemical components of LSS. [8]. Proteins of LSS, among which hydrophobic proteins of surfactant system B and C (SP-B and SP-C correspondingly) and hydrophilic ones A i D (SP-A and SP-D) are distinguished provide formation of an adequate immune response in the lungs. Being components of the system of innate immunity, SP-A and SP-D in the main provide development of adequate immune response under the action of components of the outer environment and pathogens, takingan active part in post-infection defense of respiratory tract [8, 9, 16]. By the data of scientific research even insignificant decrease of surfactant protein concentration D (SP-D) in the bronchial-alveolar fluid is accompanied by increase of incidence of respiratory infections, and its excess is a risk factor for development of allergic diseases of the respiratory tract [6, 9]. Absence of SP-D in the bronchial-alveolar fluid increases the risk of bacterial colonization of upper and lower ways and favors bacteremia development [6, 17].

SP-D is calcium-dependent collagen glycoprotein (lectin), which belongs to the family of so-called lectins (carbohydrate-bounding proteins). It is secreted by type II alveolocytes and by non-ciliary bronchiolar Clara's cells [7, 11, 12]. By the data of some researches, in very small amount it was revealed in endothelium and glandular cells of alimentary tract and on the surface of mucous membranes, in mammary and thyroid glands, urinary bladder, kidneys, heart, brain, uterus, lacrimal and sudoriferous glands [7, 9].

There exists a few works, concerning changes of SP-D levels in patients with various pathology, in the basis of which acute and chronic inflammation plays a definite role [11, 13, 18]. Probably, SP-D plays an important role in infectious lesion of the lungs. The latter influences on the mechanisms of interaction between macrophages and pathogens. Owing to the ability of binding with lipopolysaccharides which are present on the surface of gram-negative bacteria, SP-D is combined with *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Hemophilus influenze*. This favors their agglutination and stimulates chemotaxis of neutrophils, macrophages and eosinophils to the site of pathogen invasion [7, 9]. SP-D may combine with gram-positive bacteria, such as *Streptococcus pneumoniae* and *Staphylococcus aureus*, as well as with mycobacteria, viruses, fungi [16]. Since SP-D is a secretory protein which recognizes and binds pathogenic microorganisms, it is called «secretory receptor, which recognizes pathogens» [7, 16]. So, SP-D serves as a marker of pathogenic microorganisms, which are dedicated to be destructed by the immune system and as an attractant for the immune cells, i.e. increases efficacy of phagocytosis [6, 16].

Thus and so, decrease of SP-D content in the lungs leads to increase of organism's sensitivity to infections [6]; this was confirmed experimentally on SP-D (-/-) mice, which were more sensitive to respiratory tract infections [7, 11].

By the opinion of some researches, SP-D like the protein LSS may be a local analog of proteins of acute phase, level of their concentration sharply grows in the acute period of disease [10, 13, 17].

In this connection the aim of our work was assessment of diagnostic significance of SP-D protein in patients with acute infectious pulmonary diseases and exacerbation of chronic pathology, namely with community acquired pneumonia (CAP) and infectious exacerbation of chronic obstructive pulmonary disease COPD) with further defining of specificity for each process separately.

2. Materials and methods

Patients with diagnosed CAP and infectious exacerbation of COPD undergoing inpatient treatment in clinics of Dnipropetrovsk were enrolled in the investigation. Diagnoses were established according to the Order № 128 of HM of Ukraine from 19.03.2007 and according to GOLD criteria (2008). Patients on antibacterial medication lasting more than 24 hours were not enrolled in the study.

All patients underwent general clinical investigations, roentgenography of chest organs in two projections and according to ATS/ERS standards [14], external respiration (ER) was assessed by means of spirograph MasterLab (Viasis, Germany). Microbiologic investigation was performed in the laboratory of diagnostic center of CLL «Pharmacies of medical academyї» city of Dnipropetrovsk.

Furthermore, SP-D and C-reactive protein (C-RP) level in blood plasma were measured in patients and in persons of control group, composing research population. Plasma level of SP-D was defined by means of immune-ferment analysis using reagents «Hycult Biotech» (Netherland) in accordance with protocol of manufacturer. Plasma level of C-RP was defined with the help of IT-CRP 2 * 1S reagents (Lachema, Slovakia) with immunoturbidimetric method using photometer «Microlab-200» in accordance with protocols of manufacturer.

The results obtained were processed by means of program «Microsoft Office Excel» and «Statistica 6» using non-parameter statistic methods. Mann-Whitney's U-test was used for comparison of groups. Dependence between variables was assessed by means of Spirman's correlation ratio. Variations of $p < 0,05$ between findings in the groups were considered to be significant.

3. Results and discussion

55 patients with CAP and infectious exacerbation of chronic obstructive pulmonary disease COPD) on antibacterial therapy (ABT) lasting not more than one day were enrolled in the research. 10 healthy persons enrolled in the research made up control group. Patients were divided into two groups in accordance with diagnosis established.

35 patients with established CAP diagnosis, median age of 48,6 (35,0-62,0) years composed the first group; of them there were 19 males and 16 females, percentage ratio being 54% to 46% correspondingly.

20 patients with established COPD diagnosis in the stage of infectious exacerbation, median age of 68,7 (53,0-67,0) years composed the second group, of them there were 13 males and 7 females, percentage ratio being 65% to 35% correspondingly.

10 practically healthy persons, median age of 32,7 (24,0 – 46,0) years composed group of comparison of them there were 7 males and 3 females, percentage ratio being 70% to 30% correspondingly.

Characteristic features of the basic clinical symptoms of both groups are presented in the table 1.

In patients of both groups biochemical blood indices (activity of alanine aminotransferase (ALT) were within the norm.

Table 1: Clinical characteristics of patients of the 1-st and 2-nd groups

Index	1-st group N=35	2-nd group N=20
Age, years	48,6 (35,0-62,0)	68,7 (53,0-67,0)
Clinical characteristics		
Body temperature: – normal, % – subfebrile, % – febrile, %	 – 42 58	 20 80 –
Breathing rate per minute	24,3±2,8	22,8±2,4
Pulse	87,2±5,9	79,5±4,8
Breathlessness, %	51	100

Cough: Productive, % Dry, short, %	 66 34	 100
Wheezes,% Fine moist rales, %	– 66	100 –
Laboratory characteristics		
Leukocytes, 10^{-9}/ml	12,8 ±4,9	9,5 ±2,8
Rod-shaped neutrophils, %	10,6±3,9	8,3±3,2
ESR, mm/h	26,1±3,4	14,7±4,2
Sputum discharge: Purulent, % Mucopurulent, % Mucoid, %	N=23 32 68 11	N=20 35 65 –

Measuring of plasma level of C-reactive protein C-RP and SP-D was performed in patients of both groups and those of control one. Results are presented in the tables 2 and 3.

Table 2: Inflammatory markers in patients of the 1-st group and healthy persons

Indices	Patients of 1-st group	Healthy persons (3)
SP-D, Me [25-75%] ng/ml	452,70 [372,04-624,32]	240,00 [164,74-380,00]
C-RP, Me [25-75%] g/l	81,13 [28,84-200,80]	5,49 [5,42-5,59]

In patients of the first group evidence of inflammatory process in the lungs on roentgenogram in 91% of cases was unilateral. The process was localized within the limits of some segments of one portion in 14% of cases, within the limits of one portion in 80% of cases, within the limits of two portions in 6% of cases. Bilateral infiltration in patients of the first group was revealed in 9% of cases.

Of 35 patients of the first group only in 19 (54%) there was observed discharge of purulent and mucopurulent sputum, in 4 (11%) cases samples were recognized as non-representative. Of 19 patients causative agent of the disease was verified in 7 cases, making up 37%. In four cases *H. influenzae* strains were revealed, in two – *H. parainfluenzae* and in one case *S. pneumoniae* was etiologic causative agent. Herewith, pathogenic microorganisms were revealed in diagnostically significant concentration.

In general clinical analysis signs of systemic inflammatory process, represented by increased count of leukocytes, rod-shaped neutrophils and ESR were noted. C-RP was among the markers under investigation for analyzing systemic inflammatory response, level of its indices was reliably higher in patients of the first group than in healthy persons ($p< 0,001$) and on average increased up to 81,13 g/l, while maximal marks reached 405 g/l. However under the action of anti-inflammatory cytokines C-RP synthesis grows already in 6 hours from bacterial infection development, and concentration of it increases by 10 – 100 times during 24-48 hours [4, 15]. The highest levels were observed in patients with a high degree of manifestation of clinical and laboratory symptoms, this characterized the degree of manifestation of organism's systemic inflammatory response to invasion of pathogenic microbe agent.

By statistical data SP-D level in the 1-st group made up 452,70 [372,04-624,32] ng/ml, being reliably higher than in control group ($p=0,002$). Maximal values of SP-D in patients of the first group were 827,35 ng/ml in a patient with bilateral lesion of the lungs, much higher values were in patients with lesion of some portions in one side; this indicated to a more marked area of lesion and bacterial infiltration.

In patients of the second group in 65% of cases there was observed the first type of exacerbation by Anthonisen N.R. [5] and in 35% – second one. According to expressiveness of symptoms exacerbation fell into moderate degree of severity in 95% of patients, while in 5% – into severe degree. By the data of spirometry, FEV1 index was defined at the level of 48,4 [39,8 – 64,3] percent.

Analyzing bacteriologic data of sputum of second group patients, all samples proved to be representative, etiologic agent was revealed more often than in patients of the first group and was verified in 45% (9) cases. The cause of this infectious exacerbation was *H. influenzae* 45% (4), *M. catarrhalis* 22% (2), *Kl. pneumoniae* 22% (2), *P. aeruginosa* 11% (1). Identified causative agents had diagnostically significant concentration of colony-forming units. In patients with verified causative agent a more severe course of exacerbation was noted in patients with *Kl. pneumoniae* and *P. aeruginosa*, while in patients with *H. influenzae* intensity of clinical symptoms was less, course of the disease was of moderate severity.

By the data obtained C-RP level in patients of the second group was reliably higher than in healthy persons ($p< 0,001$), the highest index of C-RP was noted in the patient of 2-nd group with 1 type of exacerbation by Anthonisen N.R. [5] making up 66 g/l, herewith, pronounced manifestation of clinical and laboratory symptoms was observed. Among these patients there were observed cases of fixing of levels within normal values, which did not exceed 10 g/l, however not lower than 8 g/l, this in highly sensitive range may characterize chronic course of the process with moderate level of bacterial loading.

Table 3: Inflammatory markers in patients of 2-nd group and healthy persons

Indices	Patients of 2nd group	Healthy persons (3)
SP-D, Me [25-75%] ng/ml	553,38 [406,26-747,54]	240,00 [164,74-380,00]
C-RP, Me [25-75%] g/l	14,41 [6,39-41,25]	5,49 [5,42-5,59]

Having analyzed the data concerning other marker, it was noted that increase of SP-D level made up 553,38 [406,26-747,54] ng/ml on average, this was reliably higher than in healthy persons ($p< 0,001$). One of the highest level of SP-D index was noted at the mark of 1429,73 ng/ml, revealed in the

patient with 1 type of exacerbation by Anthonisen N.R. [5] and in the context of the disease may indicate to a high degree of intensity of bacterial loading in exacerbation of chronic inflammatory process in the lungs, taking into account lesion of a considerable area of lung surface.

By the results of microbiologic studies in both groups 37% of identified microorganisms were in the first group against 45% in the second group. Herewith, in the first group only respiratory pathogens were verified, which belong to the main causative agents of lower respiratory tract infections [1, 2], while in the second group 33% of isolated pathogens were related to «problem» strains [2, 3]. However, among revealed pathogenic microorganisms resistant to antibacterial agents, strains were not revealed.

C-RP indices of the first and second group were reliably higher in patients of the first group ($p< 0,001$), and in this context it should be mentioned that C-RP is not specific namely for COPD, but this parameter is identified more often than other laboratory markers of activity of the inflammatory process. In clinical practice C-RP makes it possible to differentiate from usual COPD exacerbation, indicating to intensity of systemic inflammatory response [11].

By the obtained results, concerning levels of SP-D markers, it should be noted that average indices in patients of the second group were by 18% higher than those of the first group, but they did not have reliable difference against each other ($p=0,144$). In this particular case marker reflects degree of pulmonary tissue lesion resulted from impact of infectious agents. SP-D index in patients of the second group is linked with bigger area of affection and chronic course of the inflammatory process in a stable phase of the disease, while patients of the first group had less area of focal affection and acute course of the process with further spread of inflammation.

Taking into account that SP-D indirectly is responsible for lipid homeostasis, increases uptake of pathogens by alveolar macrophages, activating local anti-infectious pulmonary immunity with enhanced SPD synthesis to the response on the impact of microbe agents and development of inflammatory process in the lungs [6, 9], it was expedient to investigate changes of marker levels in dependence with identification of microbe agent.

By the obtained results there were no reliable differences between patients of the first group with identified infectious agent and in patients without identified microorganism ($p=0,658$). The same situation was observed in patients of the second group ($p=0,835$). There was no reliable difference in patients with Gr (-) and Gr (+) infectious agents as well ($p=0,573$). Probably it was linked with insufficient number of the researches performed, however in the context of the results obtained it should be ascertained that SP-D is not the marker which in 100% may testify to etiologic significance of microbe infectious agent in general or Gr (+) or Gr(-) microorganisms separately, that is why clinical and radiological data and data of bacteriologic study of sputum are the principal ones in establishing etiologic diagnosis and administering ABT. So, SP-D is not the marker of infectious agent presence, but reflects the level of pulmonary tissue lesion.

Revealing link between C=RP and SP-D in patients with LRTI was advisable as the question if one marker is enough to define expressiveness of inflammation of pulmonary tissue arouse. Performed correlation analysis did not reveal probable link between plasma level of C=RP and SP-D in patients of the first group ($R = 0,095$, $p = 0,589$), in the same manner there was no correlation link in patients of the second group ($R =-0,047$, $p = 0,845$). By the analysis data it was not possible to accessory assess belonging of both markers to manifestation of expressiveness of local inflammation of pulmonary tissue.

In the routine doctor's practice C-RP level is being defined more often due to the rate of obtaining results and economic availability of this research method. However SP-D being more specific for LRTI, taking into account its role in non-specific immune defense of LSS and different mechanisms of reaction to invasion of alien agents through respiratory ways, it requires introduction into practical medicine for diagnostic programs, as due to the information on distribution of its concentrations between blood plasma and lavage fluid it becomes more reliably to assess functional state of the lungs and their compensatory possibilities in response to the lesion.

It should also be mentioned that in diagnostic practice it is expedient to use both markers together for more fuller characteristics of inflammatory process – C-RP as a marker of systemic inflammatory response, which is produced as a response to inflammation of tissue or development of infectious process in the organism and SP-D as a protein of LSS characterizing degree of lesion of pulmonary tissue, which develops as a result of inflammatory process.

4. Conclusions

1. Revealing of infectious agents in patients of the first and second groups was recorded in 37% and 45% of cases correspondingly. Values of SP-D levels in both groups with identification of causative agent and without it did not have reliable difference ($p=0,658$; $p=0,835$).
2. Plasma level of SP-D in patients of the first and second groups was reliably higher as compared with control group ($p=0,002$; $p< 0,001$), indicating to the presence of affected pulmonary tissue due to development of inflammatory process. However, reliable difference between findings of the first and second group was not revealed ($p< 0,144$).
3. Plasma level of C-RP was reliably higher in patients of both groups as compared with control one ($p< 0,001$), this reflects expressiveness of systemic inflammatory response, but it is not specific for each particular nosology. C-RP level was reliably higher in patients of the first group relatively the second one ($p< 0,001$).

Despite the fact that correlation link between C-RP and SP-D was not revealed, being probably connected with different specificity of these markers for infectious processes of the respiratory system; the need in their investigation remains an important step for defining expressiveness of inflammation and lesion of pulmonary tissue in patients with LRTI.

5. References

1. Дзюблик ЯО. Клінічні аспекти антибіотикорезистентності збудників негоспітальних інфекцій дихальних шляхів. Український пуль монологічний журнал 2010; 3:53-56.
2. Рачина СА, Козлов РС, Шаль ЕП. и др. Структура бактериальных возбудителей внебольничной пневмонии в многопрофильных стационарах Смоленска. Пульмонолгия 2011; 1:5-18.
3. Фещенко Ю.І. ХОЗЛ в Украине: проблемы и пути решения. Здоров'я України 2009; 9(1):3-4.
4. Фещенко ЮІ, Голубовська ОА. та ін. Негоспітальна пневмонія у дорослих осіб: етіологія, патогенез, класифікація, діагностика, антибактеріальна терапія

(проект клінічних настанов) Частина 1. Український пуль монологічний журнал 2012; 4:5-17.
5. Anthonisen NR. The British hypothesis revisited. European respiratory journal 2004; 23:657-658.
6. Hartl D, Griese M. Surfactant protein D in human lung diseases. European Journal of Clinical Investigation 2006; 36(6):423-435.
7. Forbes LR, Haczku A. SP-D and regulation of the pulmonary innate immune system in Allergic airway changes. Clinical & Experimental Allergy 2010; 40(4):547-562.
8. Sorensen GL, Husby S, Holmskov U. Surfactant protein A and surfactant protein D variation in pulmonary disease. Immunobiology 2007; 212(4-5):381-416.
9. Crouch EC. Surfactant protein-D and pulmonary host defense. Respiratory research 2000; 1:93-108.
10. Chun-Rong J, Wei Liu, Rong-Chang C. Serum surfactant protein D: Biomarker of chronic obstructive pulmonary disease. Disease Markers 2012; 32:281-287.
11. Shakooria TA, Sinb DD, Ghafoorc F, Bashirc S, Bokharia SNH. Serum surfactant protein D during acute exacerbations of chronic obstructive pulmonary disease. Disease Markers 2009; 27:287-294.
12. Winkler C, Atochina-Vasserman EN, Holz O, Beers MF. Comprehensive characterisation of pulmonary and serum surfactant protein D in COPD. Respiratory Research 2011, 12:29.
13. Leth-Larsen R, Nordenbaek C, Tornoe I. Surfactant protein D (SP-D) serum levels in patients with community-acquired pneumonia. Clinical Immunology 2003;108(1):29-37.
14. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R. SERIES ‘‘ATS/ERS TASK FORCE: standardisation of lung function testing’’. Interpretative strategies for lung function tests. European Respiratory Journal 2005; 26:948-968.
15. Capelastegui A, España PP, Bilbao A. Etiology of community-acquired pneumonia in a population-based study: link between etiology and patient characteristics, process-of-care, clinical evolution and outcomes. BMC Infectious Diseases 2012; 12:134.
16. Van de Wetering KJ, Van Eijk M. Van Golde L. MG, Hartung T, Van Strijp JAG, Batenburget JJ. Characteristics of Surfactant Protein A and D Binding to Lipoteichoic Acid and Peptidoglycan, 2 Major Cell Wall Components of Gram-Positive Bacteria. The Journal of Infectious Diseases 2001; 184(9):1143-1151.
17. Gaunsbaek MQ, Rasmussen KJ, Beers MF, Atochina-Vasserman EN, Hansen S. Lung Surfactant Protein D (SP-D) Response and Regulation During Acute and Chronic Lung Injury. Lung 2013; 191:295-303.
18. Eisner MD, Parsons P, Matthay MA, Ware L, Greene K. Plasma surfactant protein levels and clinical outcomes in patients with acute lung injury. Thorax 2003; 58:983–988.
19. Woodhead M, Blasi F, Ewig S, Garau J. Guidelines for the management of adult lower respiratory tract infections. Clinical microbiology and infection 2011; 17(6):1-59.
20. Moellering RC Jr. The continuing challenge of lower respiratory tract infections. Clinical Infectious Diseases 2004; 38(4):319-321.