UDC 616.24-007.272-036.12:612.017.1

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Peculiarities of oral cavity tissue immunity in patients with chronic obstructive pulmonary disease associated with generalized periodontitis

Key words: *chronic obstructive pulmonary disease, immunological parameters of local immunity, generalized periodontitis.*

Chronic obstructive pulmonary disease (COPD), underlain by chronic inflammation, remains a major public health challenge [1].

Particular attention should be paid to a problem of combination of COPD and pathological processes of the oral cavity. Many studies have shown that periodontal disease is closely related to pathogenesis of systemic diseases and is a risk factor for COPD development [15, 19, 20].

Apart from lungs damage, COPD, in its turn, leads to significant extrapulmonary systemic effects [15] that include development of secondary generalized periodontitis [19]. Chronic inflammation in the bronchi reduces general immunological reactivity of the body, leads to disruption of the mechanisms of resistance of the mucous membrane both of the bronchi and of the oral cavity, which contributes to the progression of inflammation in periodontal tissues. A vast contact surface of the mucous membranes (200–330 m^2 or more) provides for continuous and intense aggression of the macro- and microenvironment. Protection of the mucous membranes of the oral cavity is performed by means of protective mechanisms of specific and non-specific nature and specialized structures.

In recent decades, an opinion was formed that the main humoral protective factors of the mucous membranes is immunoglobulin M, secretory immunoglobulin A (sIgA) and a number of protein-carbohydrate compounds – proteases and antiproteases of saliva, lysozyme, lactoferrin, mucus glycoproteids, etc. However, a number of studies have demonstrated that the subnormal formation of antibodies – the key specific protective immunity factor – is a consequence and the integral manifestation of disorder in quantitative composition of cells and their functional characteristics in immunogenesis processes, including lymphoid and non-lymphoid cells and cytokine regulation. Cytokines formed under conditions of inflammatory process damage the periodontal tissue and lead to resorption of alveolar bone, which causes further activation of their synthesis by the immune competent cells and results in chronization of the inflammation [3, 5]. Therewith, in periodontal diseases, the most harmful effect in exerted by IL-1 [3, 14, 20].

Thus, estimation of levels of secretory immunoglobulin sIgA, total protein and concentrations of proinflammatory cytokine IL-1β is a crucial aspect in emergence of immunodeficiency state of mucous membranes of the oral cavity, and the study of these parameters of tissue immunity in patients with COPD associated with generalized periodontitis is topical.

The **aim of the study** was to investigate peculiarities of parameters of the oral cavity tissue immunity by estimating levels of proinflammatory cytokine IL-1β, content of sIgA and total protein in mixed saliva of patients with chronic obstructive pulmonary disease associated with generalized periodontitis.

The work was carried out at the expense of resources of the central government budget.

Materials and methods of the study

The study involved 63 patients with COPD in the phase of remission (group I), who were followed-up in clinics of State organization «National institute of phthisiology and pulmonology named after F. G. Yanovsky of the National Academy of Medical Sciences of Ukraine». This group included 22 women and 41 men aged 40 to 80 years (mean age was 63.8 ± 1.1 years). In all patients, the forced expiratory volume in the first second (FEV_1) before the bronchial spasmolytic test was (46.2 ± 2.0) %; FEV_1 / forced inspiratory vital capacity (FIVC) was (50.6 ± 1.6). FEV_1 after the bronchial spasmolytic test made up (48.8 ± 2.1) %; FEV_1 / FIVC (51.6 ± 1.6).

The selection of patients was performed according to the severity of the disease at the order of the Ministry of Healthcare of Ukraine June 27th, 2013 No. 555 «Unified clinical protocol of primary, secondary (specialized), tertiary (highly specialized) care and medical rehabilitation, «Chronic obstructive pulmonary disease» [12]. The patients were split into clinical groups based on the severity of clinical symptoms, functional indicators and risk of possible complications [15].

The control (II) group consisted of 30 apparently healthy individuals who had voluntarily agreed to participate in the study. The control group included 16 men and 9 women aged 40 to 80 years, mean age was (59.6 ± 1.3) years, FEV_1 was (111.0 ± 3.3) %; FEV_1 / FIVC – (78.0 ± 0.6).

During examination of patients of groups I and II questionnaire survey, clinical and functional, periodontal examination, multidetector computed tomography (MCT) of the maxillofacial area and immunological study were performed.

Pulmonary ventilation function was evaluated according to the data of spirogram with analysis of «flow–volume» curve of the forced expiration and whole body plethysmography using «Master Screen PFT» unit produced by company «Cardinal Health» (Germany). Before and after the bronchial spasmolytic test, FEV_1, FEV_1 / FIVC ratio were measured. The tests were conducted in the morning after a 12–14-hour break in drug exposure. To detect the presence and to evaluate the reversibility of bronchial obstruction, the respiratory function test was carried out before and in 15–30 minutes after 2 inhalations (200 μg) of short-acting β_2-agonist (salbutamol).

Periodontal examination was performed according to conventional methods by a dentist [2]. Results of the study were entered to the periodontal screening record.

MCT was performed on a CT-Scanner *Aquilion TSX-101A «Tochiba»* (Japan) using the K-Pacs program [9].

Study of tissue immunity of the oral cavity was performed in 63 patients with COPD and 25 patients of the control group.

During the immunological study of the patients, markers of local inflammation – IL-1β, sIgA and total protein – were estimated in mixed saliva of the patients. Collection of mixed saliva was performed in the morning on an empty stomach in order to maximize exclusion of the activation factors. Mixed saliva was collected by exspuition in a special-purpose vessel [17]. Levels of IL-1β were estimated by ELISA test using commercial test kits CYTOKINE, St. Petersburg, Russia [8]. Levels of sIgA were estimated by ELISA test [10] using commercial test kits "Xema-Medica", Moscow, Russia [9]. Levels of total protein were estimated by the Folin's Lowry method [6] and by analysis of results on the spectrophotometer μQuant (BioTek, USA). The obtained results were compared with the values received in the control group and with the reference values of content of IL-1β, sIgA and total saliva protein obtained from the literature [7–10, 13, 16–18].

The digital material received in the course of the study in each sample was checked and confirmed for normal distribution of values. To test the normality of the data distribution, method of S. N. Lapach et al. was employed (2001) (feature NORMSAMP-1, which is embedded in the Excel environment). Based on the obtained results, method of further statistical procession of data was selected to confirm the probability of the results [11].

To assess the reliability of differences between mean values of parameters in samples with normal distribution, Student's paired t-test was employed (for dependent and independent samples). The index value of probability (p-value) between the groups, which equaled or was less than 0.05, was adopted as the probability level. In the absence of normality of distribution, Wilcoxon two-sample test was employed for calculation of probability of differences between mean values. In this case, the assessment was performed by comparison of maximal and minimal criteria values. During analysis of individual changes in the studied parameters, alternative method variation was employed.

Storage of the study results and their mathematical procession were carried out using licensed software products that were part of Microsoft Office Professional 2007 package, license of Russian Academic OPEN No Level № 43437596.

Results and discussion

In the course of examination of 63 patients with COPD, who were followed-up in State organization «National institute of phthisiology and pulmonology named after F. G. Yanovsky of the National Academy of Medical Sciences of Ukraine» all patients were split into clinical groups based on the evaluation of severity of clinical symptoms, functional parameters and risk of possible complications [15]. Twenty two (34.9 %) patients were allocated to clinical group B, 12 (19.1 %) – to clinical group C and 29 (46.0 %) – to clinical group D.

Thus, the major part of patients with COPD, who were being followed-up at the institute, were patients from clinical group D.

Periodontal pathology and its complications topped the structure of dental diseases in patients with COPD.

On the basis of clinical symptoms and results of periodontal examination, periodontal disease was diagnosed in all patients of the treatment group. Seventy (27.0 %) out of 63 patients of group I exhibited complete secondary edentulism which had occurred as a complication of generalized periodontitis. These patients used removable dental prostheses. Other 46 (73.0 %) patients of this group exhibited severe tooth loss and required orthopedic care.

It should be noted that among 17 patients with COPD with complete secondary edentulism, 7 (41.2 %) patients belonged to clinical group B, 2 (11.8 %) – to clinical group C. A significant proportion – 8 (47.0 %) patients – belonged to clinical group D.

None of the patients in group II had complete secondary edentulism. However, due to partial tooth loss and denture defects, 26 (66.7 %) out of 30 individuals required orthopedic treatment. That indicated that individuals in the control group required orthopedic correction, but to a much lesser extent.

Examination of patients with COPD performed by a dentist on the basis of periodontal screening and MCT reveled 1st degree generalized periodontitis in 29 (46.0 %) patients, 2nd degree generalized periodontitis in 17 (27.0 %) patients (Table 1).

All individuals in group II exhibited signs of generalized periodontitis as well, but in most cases those signs corresponded to initial or 1st degree of severity. Thus, patients in the control group were diagnosed with 1st degree generalized periodontitis – 28 (93.3 %) patients – and 2nd degree generalized periodontitis – 2 (6.7 %) patients, which corresponded to age-related changes described in the literature.

Given the fact that the control group was identical to the group of patients with COPD in terms of age and sex composition, the first stage of the work was to determine the limits of fluctuations in secretory immunoglobulin sIgA, proinflammatory cytokine IL-1β and total protein and to estimate frequency of their deviation from generally accepted reference values. The received data are presented in Table 2.

Analysis of frequency of deviations in immunological markers of local inflammation from the generally accepted norms in healthy individuals [7–10, 13, 16–18], revealed the following. The most significant deviations in individuals of the control group were those in IL-1β and sIgA content. Twenty four (96.0 ± 3.9 %) out of 25 patients demonstrated elevated levels of IL-1β; 8 (32.0 ± 9.3 %) patients-elevated levels of sIgA, while only in 2 (8.0 ± 5.4 %) patients sIgA level was lower than the generally accepted reference values. No elevations of total protein level in saliva were detected.

The examination revealed changes in immunological markers of local inflammation in mixed saliva almost in all patients with COPD. 39 (61.9 ± 6.1 %) out of 63 patients had elevated levels of IL-1β, which was significantly different from the frequency of this cytokine production increase in the control group ($p < 0.05$) according to results of Student's t-test. 19 (30.2 ± 5.8 %) patients ($p < 0.05$) had elevated levels of sIgA

Table 1

Allocation of patients of groups I and II by the pathological processes of periodontitis

Diagnosis	Group I (n = 63)		Group II (n = 30)	
	Absolute value	%	Absolute value	%
I stage generalized periodontitis	29	46.0 ±	28	93.3 ±
II stage generalized periodontitis	17	27.0 ±	2	6.7 ±
Secondary edentulism	17	27.0 ±	–	–

Note: * – difference between groups I and II is statistically significant ($p < 0.001$)

Table 2

Immunological parameters of local inflammation in the control group and in patients with COPD

Parameters	Mean value, median and fluctuation limits of saliva parameters			Percentage of patients with parameters different from the reference values	Direction of changes
	M	Me	Fluctuation limits	M ± m	
Reference values					
IL-1β, pg/ml	101.0		25.3-175.2		
sIgA, μg/ml	155.6		57.0-260.0		
Total protein, mg/ml	1.58		0.7-3.0		
Control group (n = 25)					
IL-1β, pg/ml	642.0	595.7	27.0-1532.8	96.0 ± 3.9	↑
sIgA, μg/ml	217.0	211.5	37.0-471.2	32.0 ± 9.3 8.0 ± 5.4	↑ ↓
Total protein, mg/ml	1.6	1.5	0.7-2.7	0.0 ± 0.0	↑
Patients with COPD (n = 63)					
IL-1β, pg/ml	342.9	194.1*	11.5-1681.7	61.9 ± 6.1#	↑
sIgA, μg/ml	181.4	154.0	10.1-533.0	30.2 ± 5.8 23.8 ± 5.4#	↑ ↓
Total protein, mg/ml	1.6	1.3	0.4-6.9	4.8 ± 2.7	↑

Notes: * – difference in the parameter as compared to the control was statistically confirmed by Wilcoxon rank-sum test ($p < 0.05$); # – difference in the parameter as compared to the control was statistically confirmed by Student's t-test ($p < 0.05$); ↑ – increase; ↓ – decrease - direction of changes.

Table 3

Immunological parameters of local inflammation in patients with COPD in clinical groups B, C and D

Parameters	Mean value, median and fluctuation limits of saliva parameters			Percentage of patients with parameters different from the reference values, M ± m	Direction of changes
	M	Me	Fluctuation limits		
Reference values					
IL-1β, pg/ml	101.0		25.3–175.2		
sIgA, μg/ml	155.6		57.0–260.0		
Total protein, mg/ml	1.58		0.7–3.0		
Control group (n = 25)					
IL-1β, pg/ml	642.0	595.7	27.0–1532.8	96.0 ± 3.9	↑
sIgA, μg/ml	217.0	211.5	37.0–471.2	32.0 ± 9.3 8.0 ± 5.4	↑ ↓
Total protein, mg/ml	1.6	1.5	0.7–2.7	0.0 ± 0.0	↑
Patients with COPD group B (n =22)					
IL-1β, pg/ml	335.3	112.7*	23.8–1460.4	54.5 ± 10.6#	↑
sIgA, μg/ml	217.9	177.3D	10.1–533.0	40.9 ± 10.5D 27.3 ± 9.5	↑ ↓
Total protein, mg/ml	1.7	1.3	0.8–6.9	8.3 ± 8.0	↑
Patients with COPD group C (n = 12)					
IL-1β, pg/ml	393.2	210.5*	11.5–1499.5	75.0 ± 12.5	↑
sIgA, μg/ml	205.5	231.6	13.5–378.1	41.7 ± 14.2 25.0 ± 12.5	↑ ↓
Total protein, mg/ml	1.7	1.2	0.4–5.3	4.5 ± 4.4	↑
Patients with COPD group D (n = 29)					
IL-1β, pg/ml	327.8	198.7*	11.5–1681.7	62.1 ± 9.0#	↑
sIgA, μg/ml	143.7	121.9*B	10.1–366.4	17.2 ± 7.0B 20.7 ± 7.5	↑ ↓
Total protein, mg/ml	1.5	1.4	0.5–3.2	4.8 ± 2.7	↑

Notes: B, C, D – difference in the parameter between the groups was statistically confirmed by Wilcoxon rank-sum test ($p<0.05$); B, C, D – difference in the parameter between the groups was statistically confirmed by Student's t-test ($p<0.05$) * – difference in the parameter as compared to the control was statistically confirmed by Wilcoxon rank-sum test ($p<0.05$) # – difference in the parameter as compared to the control was statistically confirmed by Student's t-test ($p<0.05$) ↑ – increase; ↓ – decrease - direction of changes.

and 15 (23.8 ± 5.4 %) of patients ($p < 0.05$) had reduced levels of sIgA, which exceeded frequency of this marker reduction in the control group 3-fold ($p < 0.05$) according to results of Student's t-test. Increased levels of total protein were estimated only in 3 patients (4.8 ± 2.7 %).

Estimation of concentrations of inflammatory markers in patients with COPD and apparently healthy individuals of the same age and sex showed a significant reduction in IL-1β content in mixed saliva to 194.1 pg/ml (fluctuation limits: 11.5–1681.7 pg/ml) in patients of the treatment group as opposed to the control group, where concentrations of IL-1β were 595.7 pg / ml (fluctuation limits: 27.0–1532.8 pg / ml), which was 3.8 times lower than the reference values of this parameter ($p < 0.05$) according to results of Wilcoxon rank-sum test. This can be explained by use of inhaled corticosteroids that had significant anti-inflammatory and immunosuppressive effects in the basic treatment of patients with COPD. Besides, an insignificant decrease in concentrations of sIgA to 154.0 μg/ml (fluctuation limits: 10.1–533.0 μg/ml) was observed in patients with COPD as opposed to the control group which was due to the fact that a larger number of patients exhibited decreased sIgA levels as compared to the control group.

Based on the severity of clinical symptoms, functional parameters and risk of possible complications, it appeared that most patients in clinical groups B, C and D demonstrated: statistically significant reduction of IL-1β in mixed saliva and changes from normal concentration of sIgA in group B to

descent of antibody formation in group D, which indicated reduction in the ability to generate an adequate response to microbial antigens and metabolic disorders depending on the severity of the disease. The data are presented in Table 3.

The lowest concentrations of IL-1β and sIgA in saliva were determined in patients of clinical group D, which was characterized by severe clinical symptoms, low values of functional parameters and the highest risk of potential complications of COPD.

Concentration of IL-1β in patients of clinical group D reached 198.7 pg/ml (fluctuation limits: 11.5–1681.7 pg/ml), which was statistically significantly different from this parameter in clinical group B – 112.7 pg / ml (fluctuation limit: 23.8–1460.4 pg/ml), $p < 0.05$ according to results of Wilcoxon rank-sum test. In group C this parameter had an intermediate value between group B and D values and made up 210.5 pg/ml (fluctuation limits: 11.5–1499.5 pg/ml).

Analysis of frequency of increase of IL-1β in the clinical groups revealed the highest frequency in group C (75.0 ± 12.5 % of cases) as compared with clinical groups D and B, but these data were not significantly different among themselves.

Analysis of data on concentration of sIgA in clinical groups of patients with COPD revealed that patients of clinical group D had significantly lower levels of this immunoglobulin than the apparently healthy individuals. Patients of clinical groups C and B were not significantly different from the apparently healthy individuals by values of sIgA saliva concentration, but a significant reduction in this parameter was clearly traced depending on the severity of the disease, from clinical group B to clinical group D (from 177.3 μg/ml to 121.9 μg/ml, respectively). Levels of total protein between the groups were almost similar.

Analysis of frequency of increase in sIgA in clinical groups revealed the highest frequency in groups C and B (40.9 ± 10.5 % and 41.7 ± 14.2 % of cases, respectively) as compared with clinical group D (17.2 ± 7.0 % of cases), $p < 0.05$ according to results of Student's t-test. This may also indicate a high risk of adverse events of course of generalized periodontitis and COPD in clinical group D. Analysis of frequency of detection of reduction in sIgA showed no significant differences between the groups.

Conclusions

It was established that in patients with COPD of clinical groups B, C and D, the major dental disease was periodontal pathology which was clinically manifested by symptoms of I–II degree generalized periodontitis and its complications – partial or complete secondary edentulism that apart from sanation of oral cavity required extensive orthopedic care specific for that category of patients.

Patients of clinical group D exhibited marked clinical symptoms of generalized periodontitis, presence of its complications – complete secondary edentulism – and the lowest levels of immunological markers of inflammation in mixed saliva of IL-1β and sIgA, which was an unfavorable factor of the course of generalized periodontitis and COPD. This indicated a reduced ability to generate an adequate immune response to microbial pathogens of the oral cavity which led to colonization of the mucous membrane of the bronchopulmonary system by those pathogens.

Additional adverse factor that suppressed the immune response in COPD was the use of inhaled corticosteroids that, on the one hand, had a pronounced anti-inflammatory effect, and on the other hand, inhibited antibody formation and synthesis of essential factors of local protection.

It was established that in the studied patients, deviations in immunological parameters of inflammation in mixed saliva were both due to COPD and generalized periodontitis and age-related changes in the body, which had been demonstrated by the results of control studies in apparently healthy individuals of the respective age category (40 to 80 years).

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ОСОБЕННОСТИ МЕСТНОГО ИММУНИТЕТА РОТОВОЙ ПОЛОСТИ У БОЛЬНЫХ ХРОНИЧЕСКИМ ОБСТРУКТИВНЫМ ЗАБОЛЕВАНИЕМ ЛЕГКИХ В СОЧЕТАНИИ С ГЕНЕРАЛИЗОВАННЫМ ПАРОДОНТИТОМ

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Резюме

Заболевания пародонта тесно связаны с патогенезом системных заболеваний, в том числе являются фактором риска развития хронического обструктивного заболевания легких (ХОЗЛ). Многими исследованиями доказано, что определение уровня секреторного иммуноглобулина А (sIgA), количества общего белка и концентрации провоспалительного цитокина IL-1β является решающим фактором выявления иммунодефицитного состояния слизистой оболочки ротовой полости.

Цель *исследования: изучить особенности показателей местного иммунитета полости рта путем определения уровня провоспалительного цитокина IL-1β, содержания sIgA и общего белка в смешанной слюне больных ХОЗЛ, сочетающимся с генерализованным пародонтитом.*

Материалы и методы исследования. *Обследовано 63 больных ХОЗЛ и 30 практически здоровых лиц в возрасте от 40 до 80 лет, которым проводились анкетирование, клинико-функциональное и пародонтологическое обследование, многосрезовая компьютерная томография (МСКТ) челюстно-лицевой области и иммунологическое исследование.*

Результаты и их обсуждение. *Установлено, что у больных ХОЗЛ клинических групп В, С и D ведущее место среди стоматологических заболеваний занимает патология пародонта, что клинически проявляется симптомами генерализованного пародонтита I–II степени тяжести и его осложнениями – вторичной адентией, что требует кроме санирующей терапии ротовой полости широкого применения ортопедической помощи у данной категории больных.*

Дополнительным неблагоприятным фактором, который подавляет иммунный ответ при ХОЗЛ, является применение ингаляционных кортикостероидов, которые, с одной стороны, обладают выраженным противовоспалительным действием, с другой – подавляют антителообразование и синтез необходимых факторов местной защиты.

Выводы. *Установлено, что наиболее низкие концентрации иммунологических показателей воспаления в смешанной слюне IL-1β и sIgA выявляются в клинической группе D, что является неблагоприятным фактором течения генерализованного пародонтита и ХОЗЛ, поскольку свидетельствует о снижении возможности формирования адекватного иммунного ответа на микробные антигены ротовой полости, что, в свою очередь, приводит к колонизации патогенами бронхолегочной системы.*

У исследуемых больных отклонения иммунологических показателей воспаления в смешанной слюне связаны как с заболеванием ХОЗЛ и генерализованным пародонтитом, так и с возрастными изменениями организма, что продемонстрировали результаты контрольных исследований у практически здоровых лиц соответствующей возрастной категории (от 40 до 80 лет).

Ключевые слова: *хроническое обструктивное заболевание легких, иммунологические показатели местного иммунитета, генерализованный пародонтит.*

Научно-практический журнал «Астма и Аллергия», 2014, № 2

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FEATURES OF ORAL CAVITY LOCAL IMMUNITY IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE COMBINED WITH GENERALIZED PERIODONTITIS

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Abstract

Periodontal disease is closely related to the pathogenesis of systemic diseases, including a risk factor for chronic obstructive pulmonary disease (COPD). Many studies have shown that the determination of secretory immunoglobulin sIgA, total protein amount and concentration of proinflammatory cytokine IL-1β, is a crucial factor in establishing immunodeficiency state of the mucous membranes of the oral cavity.

The aim *– to explore the features of the performance of local immunity in oral levels of proinflammatory cytokines IL-1β, containing sIgA and total protein in mixed saliva of patients with chronic obstructive pulmonary disease combined with generalized periodontitis.*

Materials and methods. *The study involved 63 patients with COPD and 30 healthy individuals aged 40 to 80 years who underwent: questionnaire, clinical, functional, periodontal examination, multislice computed tomography (MSCT) maxilla - facial area and immunological studies.*

Results. *It was found that patients with COPD clinical groups B, C and D leading place among the ranks of dental diseases periodontal pathology, clinically manifested symptoms of generalized periodontitis - II severity and its complications - secondary adentia requiring treatment except sanifying oral widespread use of orthopedic care given patients.*

An additional adverse factor that suppresses the immune response in COPD is the use of inhaled corticosteroids, which on the one hand have a pronounced anti-inflammatory effect, and on the other hand inhibit antibody synthesis and essential factors of local protection.

Conclusions. *Established that the lowest concentration of immunological markers of inflammation in the mixed saliva IL-1β and sIgA detected in the clinical group D, which is an unfavorable factor of generalized periodontitis and COPD, as evidenced by a decrease in the possibility of forming an adequate immune response to microbial antigens of the oral cavity, in turn leads to the colonization of pathogens bronchopulmonary system.*

In the studied patients deviation of immunological parameters in mixed saliva inflammation related diseases in both COPD and generalized periodontitis and age-related changes in the body, which showed the results of control studies in healthy individuals age group (40 to 80 years).

Key words: *chronic obstructive pulmonary disease, immunological parameters of local immunity, generalized periodontitis.*

Theoretical and practical J. «Asthma and allergy», 2014, 2

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