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QUESTIONABLE RELIABILITY OF HOMOCYSTEINE AS THE METABOLIC MARKER FOR FOLATE AND VITAMIN B_{12} DEFICIENCY IN PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE

NEIZVESNA POUZDANOST HOMOCISTEINA KAO METABOLIČKOG MARKERA ZA NEDOSTATAK FOLATA I VITAMINA B_{12} KOD PACIJENATA SA HRONIČNOM OPSTRUKTIVNOM BOLEŠĆU PLUĆA

Anđelo Beletić[1], Duško Mirković[1,2], Aleksandra Dudvarski-Ilić[3,4], Branislava Milenković[3,4], Ljudmila Nagorni-Obradović[3,4], Valentina Đorđević[5], Svetlana Ignjatović[1,2], Nada Majkić-Singh[6]*

[1]Center for Medical Biochemistry, Clinical Center of Serbia, Belgrade, Serbia
[2]Faculty of Pharmacy, University of Belgrade, Belgrade, Serbia
[3]School of Medicine, University of Belgrade, Belgrade, Serbia
[4]Clinic for Lung Diseases, Clinical Center of Serbia, Belgrade, Serbia
[5]Institute of Molecular Genetics and Genetic Engineering, University of Belgrade, Belgrade, Serbia
[6]Society of Medical Biochemists of Serbia, Belgrade, Serbia

Summary

Background: An increased homocysteine (Hcy) concentration may represent a metabolic marker of folate and vitamin B_{12} deficiency, both significant public health problems. For different reasons, patients with chronic obstructive pulmonary disease (COPD) are prone to these deficiencies. The study evaluates the reliability of Hcy concentration in predicting folate or vitamin B_{12} deficiency in these patients.
Methods: A group of 50 COPD patients (28 males/22 females, age ($\bar{x}\pm$SD=49.0±14.5) years was enrolled. A chemiluminescent microparticle immunoassay was applied for homocysteine, folate and vitamin B_{12} concentration. Kolmogorov-Smirnov, Mann-Whitney U and χ^2 tests, Spearman's correlation and ROC analysis were included in the statistical analysis, with the level of significance set at 0.05.
Results: Average (SD) concentrations of folate and vitamin B_{12} were 4.13 (2.16) μg/L and 463.6 (271.0) ng/L, whereas only vitamin B_{12} correlated with the Hcy level (P=–0.310 (R=0.029)). Gender related differences were not significant and only a borderline significant correlation

Kratak sadržaj

Uvod: Povišena koncentracija homocisteina (Hcy) može predstavljati metabolički marker nedostatka folata i vitamina B_{12}, značajnih problema javnog zdravlja. Bolesnici sa hroničnom opstruktivnom bolešću pluća (HOBP) skloni su nedostatku ovih vitamina usled različitih razloga. Prikazana studija procenjuje pouzdanost koncentracije Hcy kao prediktora nedostatka folata i vitamina B_{12} kod ovih bolesnika.
Metode: Studija je sprovedena u grupi od 50 osoba obolelih od HOBP (28 muškaraca/22 žene, starosti ($\bar{x}\pm$SD= 49,0±14,5) godina. Koncentracije Hcy, folata i vitamina B_{12} su određivane hemiluminiscentnim imunoodređivanjem na mikročesticama. Statistička analiza je uključila testove Kolmogorov-Smirnov, Mann-Whitney U and χ^2, Spearman-ovu korelaciju i ROC analizu, uz nivo značajnosti od 0,05.
Rezultati: Prosečne (SD) koncentracije folata i vitamina B_{12} su iznosile 4,13 (2,16) μg/L i 463,6 (271,0) ng/L, pri čemu je samo kod vitamina B_{12} uočena korelacija sa nivoom Hcy (R=–0,310 (P=0,029)). Koncentracije vita-

Address for correspondence:

Anđelo Beletić
Center for Medical Biochemistry Clinical Center of Serbia
Višegradska 26
11000 Belgrade, Serbia
Phone: + 381 60 15 11 083
e-mail: andjelo.beletic@yahoo.com

List of abbreviations: Hcy, homocysteine; Met, methionine; HHcy, hyperhomocysteinemia; COPD, chronic obstructive pulmonary disease; GOLD, Global Initiative for Chronic Obstructive Lung Disease; CMIA, chemiluminescent microparticle immunoassay; SD, standard deviation; Q1, 1st quartile; Q3, 3rd quartile; AUC, area under the curve; SE, standard error; CI, confidence interval.

between age and folate was confirmed (R=0.279 (P=0.047)). The incidence of folate and vitamin B_{12} deficiency differed significantly (P=0.000 and P<0.000 for folate and vitamin B_{12} respectively), depending on the cut-off used for classification (4.4, 6.6 and 8.0 μg/L – folate; 203 and 473 ng/L – vitamin B_{12}). ROC analyses failed to show any significance of hyperhomocysteinemia as a predictor of folate or vitamin B_{12} deficiency.

Conclusion: Reliability of the Hcy concentration as a biomarker of folate or vitamin B_{12} depletion in COPD patients is not satisfactory, so their deficiency cannot be predicted by the occurrence of HHcy.

Keywords: homocysteine, folate, vitamin B_{12}, COPD

mina se nisu razlikovale između polova, a starost je bila u korelaciji samo sa nivoom folata (R= 0,279 (P=0,047)). Incidenca nedostatka vitamina značajno se razlikovala (P=0,000 i P<0,000 za folat odn. vitamin B_{12}) u zavisnosti od *cut-off* vrednosti u odnosu na koju je definisana (4,4; 6,6 i 8,0 μg/L – folat; 203 i 473 ng/L – vitamin B_{12}). ROC analizom nije bilo moguće dokazati značaj hiperhomocisteinemije kao prediktora deficijencije.

Zaključak: Pouzdanost koncentracije Hcy kao markera nedostatka folata ili vitamina B_{12} kod bolesnika sa HOBP nije zadovoljavajuća, pa se deficijencija ovih vitamina ne može predvideti na osnovu pojave hiperhomocisteinemije.

Ključne reči: homocistein, folat, vitamin B_{12}, HOBP

Introduction

Folate is essential for »one carbon units' metabolism«, which generates intermediates necessary for nucleic acid synthesis and methylation of various biomolecules (1). Vitamin B_{12} is required as the coenzyme to methylmalonyl-CoA mutase (EC 5.4.99.2) and methionine synthase (EC 2.1.1.13), whereby the second enzyme links the metabolism of these two »B complex« vitamins (2). It catalyzes the conversion of homocysteine (Hcy) to methionine (Met) by virtue of a methyl group transfer from the methyltetrahydrofolate. Deficiency of either vitamin can slow the reaction and lead to deficient DNA synthesis, hyperhomocysteinemia (HHcy) and compromised methylation potential (3).

World Health Organization (WHO) estimated that folate and vitamin B_{12} deficiencies could be encountered in millions of people throughout various populations (3–5). Either of them may occur in subclinical form, when vitamin depletion can be indicated only through laboratory tests, and severe form in which clinical symptoms become overt (3). In a majority of cases, macrocytic anemia can be attributed to severe folate and vitamin B_{12} deficiencies (6). A strong causative relation has been established between severe folate deficiency and neural tube defects, adverse pregnancy outcomes, cognitive disorders in elderly etc. Severe deficiency of vitamin B_{12} is evidenced as a significant risk factor for developmental delay in childhood, neuropathy in adults and memory and cognitive impairments in all ages (4, 7). Although associated with cardiovascular disease, cancers and osteoporosis, the significance of subclinical deficiencies is still not reliably evaluated (5, 8). Inadequate folate intake is usually responsible for folate deficiency and malabsorption for vitamin B_{12} deficiency (5).

Reliable laboratory assessment of folate and vitamin B_{12} status is necessary to detect a deficiency, develop a strategy for supplementation and monitor its efficacy. Folate status is usually evaluated through measurement of the folate concentration in serum/plasma, indicating dietary intake, or erythrocytes, reflecting long-term status. Vitamin B_{12} concentrations in serum/plasma are considered a screening test in suspected deficiency, with holotranscobalamin measurement being recommended to improve sensitivity (5). The utilization of metabolic markers in assessment has been recommended by the WHO since 2005 (6). HHcy may serve as an indicator of both deficiencies. An increased level of methylmalonic acid is specific for vitamin B_{12} deficiency, while the fraction of »unmetabolized folic acid« might also be associated with impairment in the pathways depending on both vitamins (3, 5). The initially proposed cut-off values for the general population, based upon the occurrence of HHcy (defined as a Hcy concentration above 10 or 12 μmol/L) as an indicator of vitamin depletion, have undergone significant changes. For folate, 4.0 μg/L (4) was increased to 6.6 μg/L (3, 9, 10) or even 8.0 μg/L (11), while to consider vitamin B_{12} status as inadequate, cut-offs of 203 ng/L (3, 4) and 473 ng/L (9, 10) were used.

Patients with chronic obstructive pulmonary disease (COPD) are evidenced to be prone to folate and vitamin B_{12} deficiency, supposedly due to the severe nutritional impairments like increased basal metabolic rate, altered caloric intake, decreased body mass index accompanied with skeletal muscle loss. Furthermore, in certain populations an inverse relationship between folate food intake and COPD risk was observed. In studies investigating HHcy in these patients the relationship between Hcy concentration and vitamin intake or blood correlation was evaluated. Nevertheless, the ability of HHcy occurrence to predict folate or vitamin B_{12} deficiency was not addressed (12–15).

The study aims to provide an initial insight into the reliability of Hcy concentration measurement in COPD patients for the purpose of detecting folate or vitamin B_{12} deficiency. To achieve this, vitamin levels in this group were correlated with the Hcy concentration as well as with age and gender, the major physiological Hcy determinants (16). In addition, the possibility of various Hcy concentrations to predict folate or vitamin B_{12} deficiency in COPD patients was analyzed.

Materials and Methods

This cross-sectional study, approved by a competent Ethics Committee and conducted at the Clinic for Lung Diseases and the Center for Medical Biochemistry of the Clinical Center of Serbia, enrolled 50 COPD patients (28 males/22 females, age ($\bar{x}\pm$SD=49.0±14.5) years, diagnosed according to the GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria (17). Among them, 15 attended the outpatient department of the Clinic, while 35 were hospitalized and enrolled upon remission accomplishment. Exclusion criteria were smoking, alcohol consumption and supplementation with folic acid and/or vitamin B_{12}, as well as the presence of coronary heart disease, cerebrovascular and gastrointestinal disorders, renal insufficiency, diabetes, malignant or autoimmune disease.

Blood samples were collected into SST® II Advance tubes (BD Vacutainer, Franklin Lakes, NJ, USA) following 12 hours of overnight fasting after a light meal and serum was separated by centrifugation for 15 minutes at 1500 g (16, 18). The chemiluminescent microparticle immunoassays (CMIA) on the ARCHITECT® ci8200 Integrated System (Abbott Diagnostics, Wiesbaden, Germany) was applied for measurement of homocysteine, folate and vitamin B_{12} concentrations.

Kolmogorov-Smirnov, Mann-Whitney U tests and χ^2 tests, Spearman's correlation and ROC (Receiving Operator Characteristic) analysis were included in the statistical analysis, performed with SPSS® Statistic software version 22.0 (IBM®, New York, USA). Deficiencies of both vitamins were defined using the abovementioned different cut-offs from the literature. To test the possibility that these may be predicted by the occurrence of HHcy, ROC analyses were performed for HHcy defined as a Hcy concentration above 10, 12 and 15 μmol/L. These cut-offs were chosen because they are applied to distinguish tolerable from increased Hcy concentrations (19). P values below 0.05 were considered statistically significant.

Results

The Gaussian mode of distribution was observed for folate (P=0.200) and vitamin B_{12} (P=0.200), while this was not the case for Hcy (P=0.041). The following concentrations (mean (SD) /median (Q1–Q3)) were measured in patients enrolled: folate 4.13 (2.16) μg/L, vitamin B_{12} 463.6 (271.0) ng/L, and Hcy 13.22 (11.48–16.08) μmol/L. Correlation analyses of the variables investigated (*Table I*) revealed an inverse relationship between vitamin B_{12} and Hcy concentration as the only one significant.

Table I Correlation coefficients (R) between the investigated variables.

	Folate, μg/L	Vitamin B_{12}, ng/L
Hcy, μmol/L	– 0.138 (0.340)	– 0.310 (0.029)
Vitamin B_{12}, ng/L	0.008 (0.955)	/

P values are given in brackets

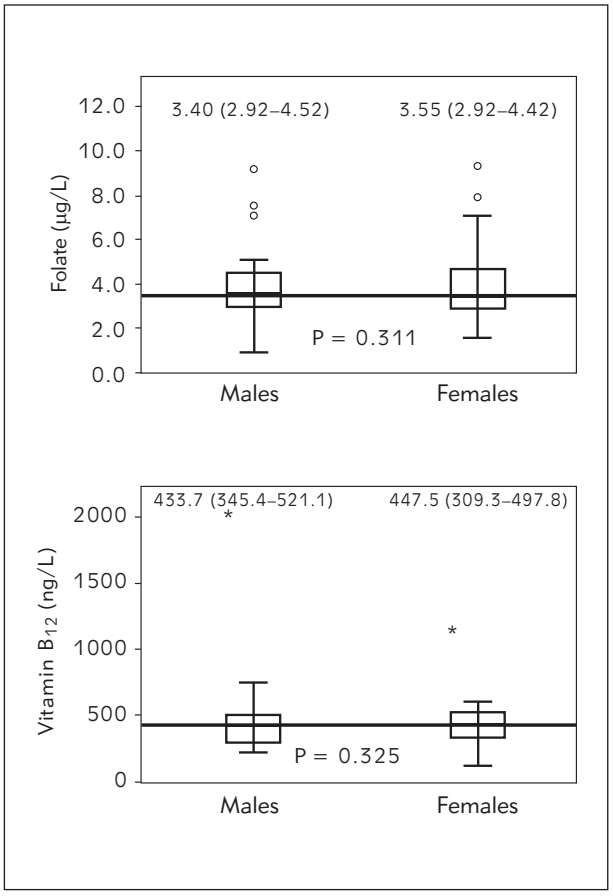

Figure 1 Gender associated differences in concentrations of folate and vitamin B_{12}.

Table II Incidence of vitamin deficiencies and the corresponding cut-offs.

Folate			
Cut-off (μg/L)	4.0	6.6	8.0
Ratio	16/50	42/50	45/50
Vitamin B_{12}			
Cut-off (ng/L)	203		473
Ratio	2/50		29/50

Table III ROC analysis for prediction of folate deficiency.

Vitamin cut-off	4.0 μg/L				6.6 μg/L				8.0 μg/L			
HHcy cut-off	AUC	SE	95% CI	P	AUC	SE	95% CI	P	AUC	SE	95% CI	P
10 μmol/L	0.514	0.101	0.317–0.711	0.721	0.552	0.112	0.332–0.773	0.651	0.686	0.109	0.472–0.900	0.229
12 μmol/L	0.556	0.103	0.354–0.757	0.584	0.603	0.113	0.382–0.824	0.375	0.661	0.139	0.389–0.933	0.298
15 μmol/L	0.620	0.094	0.435–0.805	0.235	0.615	0.104	0.410–0.819	0.322	0.671	0.113	0.451–0.892	0.267

Table IV ROC analysis for prediction of vitamin B_{12} deficiency.

Vitamin cut-off	203 ng/L				473 ng/L			
HHcy cut-off	AUC	SE	95% CI	P	AUC	SE	95% CI	P
10 μmol/L	0.553	0.267	0.029–1.000	0.859	0.633	0.097	0.443–0.824	0.166
12 μmol/L	0.632	0.224	0.193–1.000	0.657	0.671	0.094	0.487–0.854	0.076
15 μmol/L	0.842	0.108	0.631–1.000	0.248	0.504	0.096	0.316–0.693	0.965

Gender associated differences in concentration (*Figure 1*) were not of significance for either vitamin. Correlation between patients' age and vitamin concentration was characterized with the R=0.279 (P=0.047) for folate and R=–0.103 (P=0.477) in case of vitamin B_{12}.

The calculated incidence of folate and vitamin B_{12} deficiencies differed significantly (P=0.000 and P<0.000 for folate and vitamin B_{12} respectively), depending on which cut-off was used for classification (*Table II*).

ROC analyses failed to show any significance of HHcy (defined by various Hcy concentrations which should be exceeded) as a predictor of folate or vitamin B_{12} deficiency, regardless of the cut-off used to define vitamin deficiency. *Tables III* and *IV* contain details about the ROC analysis for folate and vitamin B_{12} deficiency, respectively.

Discussion

In the COPD patients enrolled, an inverse relationship between the concentrations of vitamins and Hcy was significant only for vitamin B_{12}. This finding is opposite to the one reported on the basis of values measured in a group of Italian patients (15) and partially concordant with the results in a group of patients from North America in whom no significant association was observed between HHcy and these vitamins' intake as estimated from a food frequency questionnaire (14). Although higher concentrations of folate were observed in healthy women (6, 10), gender related differences in COPD patients were not evaluated previously. Results from this study indicate that male and female patients have comparable values of both vitamins. Furthermore, a positive correlation between folate (6, 10) and vitamin B_{12} (10) levels and age was shown in a healthy population. However, only a borderline significant correlation with folate was confirmed in this study.

Average folate and vitamin B_{12} concentrations in this study were lower than those measured in a group of Swedish patients (7.0 μg/L and 540.0 ng/L for folate and vitamin B_{12} respectively) (12) and higher in comparison with Italian patients (2.5 μg/L and 324.5 ng/L) (15). Nevertheless, significant caution is recommended when interpreting these differences. Elderly patients were enrolled in the Swedish study and information about the methods applied for measurement was not included in the article reporting the results. In the study conducted in Italy, although the concentrations were also measured using CMIA, only patients with no COPD exacerbations 6 months prior to blood sampling were included.

Prevalence of deficiency in the present study was highly dependent on the cut-off values used. In the case of folate, it increased from 32.3%, when defined with the cut-off of 4.0 μg/L, to 84.0% or even 90.0% with the cut-off set at 6.6 μg/L and 8.0 μg/L respectively. For vitamin B_{12}, the change of cut-off from 203 ng/L to 473 ng/L was accompanied by an increase in the percentage of patients considered deficient from 4.0% to 58.0%. To consider Swedish

patients as folate deficient, the cut-off was set at 2.64 μg/L and yielded the prevalence of approximately 17%, while a vitamin B_{12} concentration of 203 ng/L classified almost 6% as deficient (12).

The ROC analyses, using different vitamin and Hcy cut-offs, clearly demonstrated that folate or vitamin B_{12} deficiency in the investigated group cannot be predicted by the occurrence of HHcy. Although this finding might significantly neglect the clinical utility of Hcy measurement in COPD patients, further consideration could offer additional standpoints. It may support the hypothesis that intensive methylation of DNA, RNA and different proteins during lung tissue repair generates high amounts of S-adenosyl-Hcy and Hcy in the lung tissue and plasma respectively (20). Despite the fact that this Hcy influx might be regarded as an epiphenomenon in COPD, not influenced by any traditional determinant of Hcy concentration (20), certain attention should be focused on the molecular and cellular oxidative damage caused by HHcy (21). Taken together with the report about the presence of increased oxidative stress at the moment of remission accomplishment after COPD exacerbation (22), it can be assumed that a certain time span is needed for the vitamins' depletion, predictable by HHcy, to develop in these patients.

A rather small number of participants is a limitation of the present study. Although the other studies addressing »B-vitamins complex« deficiency and its relationship with the Hcy level included a comparable number of patients, the results presented should be interpreted as »pilot« and further evaluation of their confidence in larger studies is recommended.

The results presented support the conclusion that the reliability of Hcy concentration as a biomarker of folate or vitamin B_{12} depletion in COPD is not satisfactory and that their deficiency cannot be predicted by the occurrence of HHcy.

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Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

References

1. Nazki FH, Sameer AS, Ganaie BA. Folate: metabolism, genes, polymorphisms and the associated diseases. Gene 2014; 533: 11–20.
2. Burtsi CA, Ashwood ER, Bruns DE, eds. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 4th ed. St. Louis, MO: Elsevier Saunders, 2006. pp. 1100–5.
3. Green R. Indicators for assessing folate and vitamin B-12 status and for monitoring the efficacy of intervention strategies. Am J Clin Nutr 2011; 94: Suppl 2: 666–72.
4. Conclusions of a WHO Technical Consultation on folate and vitamin B_{12} deficiencies. Food and Nutrition Bulletin 2008; 29: Suppl 2: 238–44.
5. McNulty H, Scott JM. Intake and status of folate and related B-vitamins: considerations and challenges in achieving optimal status. British Journal of Nutrition 2008; 99: Suppl 3: 48–54.
6. WHO. Serum and red blood cell folate concentrations for assessing folate status in populations. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2012. Available at: http://apps.who.int/iris/bitstream/10665/75584/1/WHO_NMH_NHD_EPG_12.1_eng. pdf. Accessed April 10th, 2014.
7. Jiang B, Ding C, Yao G, Yao C, Zhang Y, Ge J, et al. Intervention effect of folic acid and vitamin B_{12} on vascular cognitive impairment complicated with hyperhomocysteinemia. J Med Biochem 2014; 33: 169–74.
8. Guéant JL, Alpers DH. Vitamin B_{12}, a fascinating micronutrient, which influences human health in the very early and later stages of life. Biochimie 2013; 95: 967–9.
9. Dhonukshe-Rutten RA, de Vries JH, de Bree A, van der Put N, van Staveren WA, de Groot LC. Dietary intake and status of folate and vitamin B_{12} and their association with homocysteine and cardiovascular disease in European populations. Eur J Clin Nutr 2009; 63: 18–30.
10. Zappacosta B, Persichilli S, Iacoviello L, Di Castelnuovo A, Graziano M, Gervasoni J, et al. Folate, vitamin B_{12} and homocysteine status in an Italian blood donor population. Nutr Metab Cardiovasc Dis 2013; 23: 473–80.
11. De Bruyn E, Gulbis B, Cotton F. Serum and red blood cell folate testing for folate deficiency: new features? Eur J Haematol 2014; 92: 354–9.
12. Andersson I, Grönberg A, Slinde F, Bosaeus I, Larsson S. Vitamin and mineral status in elderly patients with chronic obstructive pulmonary disease. Clin Respir J 2007; 1: 23–9.
13. Hirayama F, Lee AH, Terasawa K, Kagawa Y. Folate intake associated with lung function, breathlessness and the prevalence of chronic obstructive pulmonary disease. Asia Pac J Clin Nutr 2010; 19: 103–9.
14. Seemungal TA, Cho Fook Lun J, Davis G, Neblett C, Chinyepi N, Dookhan C, et al. Plasma homocysteine is elevated in COPD patients and is related to COPD severity. Int J Chron Obstruct Pulmon Dis 2007; 2: 313–21.
15. Fimognari FL, Loffredo L, Di Simone S, Sampietro F, Pastorelli R, Monaldo M, et al. Hyperhomocysteinaemia

and poor vitamin B status in chronic obstructive pulmonary disease. Nutr Metab Cardiovasc Dis 2009; 19: 654–9.

16. Refsum H, Smith AD, Ueland PM, Nexo E, Clarke R, McPartlin J, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clin Chem 2004; 50: 3–32.
17. GOLD. Global Strategy for Diagnosis, Management, and Prevention of COPD. Report 2013_pdf. Available at: http://www.goldcopd.org. Accessed: January 9th, 2014.
18. Nikolac N, Supak-Smolčić V, Šimundić AM, Celap I. Croatian Society of Medical Biochemistry and Laboratory Medicine: National recommendations for venous blood sampling. Biochem Med 2013; 23: 242–54.
19. Wald DS, Law M, Morris JK. Homocysteine and cardiovascular disease: evidence on causality from meta-analysis. BMJ 2002; 325: 1202–6.
20. Veeranki S, Tyagi SC. Defective homocysteine metabolism: potential implications for skeletal muscle malfunction. Int J Mol Sci 2013; 14: 15074–91.
21. Hoffman M. Hypothesis: hyperhomocysteinemia is an indicator of oxidant stress. Med Hypotheses 2011; 77: 1088–93.
22. Stanojković I, Kotur-Stevuljević J, Milenković B, Spasić S, Vujić T, Stefanović A, et al. Pulmonary function, oxidative stress and inflammatory markers in severe COPD exacerbation. Respir Med 2011; 105: Suppl 3: 31–7.

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