The Role of Inflammatory Biomarkers in the Management of Children with Asthma

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Abstract

Background

Making decision on asthma as an inflammatory disease is done mostly on the airways function and the patient symptoms which most of them are nonspecific and subjective. Also, the children are not able to express their signs and collaboration in examination of the lungs function. Evaluation of serum level inflammatory biomarkers can be useful in assessment of the response to treatment and severity of asthma. In this regard, we aimed to examine the changes of serum levels of biomarkers which may occur as a result of treatment in children with asthma.

Materials and Methods

This study was conducted in 2017 and 54 children who referring to the respiratory clinic of Tabriz University of Medical Sciences, Tabriz city (Iran), were studied by convenience sampling method. To evaluate the changes of biomarkers (Immunoglobulin E, Eosinophil, Periostin and Eosinophil Cationic Protein), 5ml peripheral blood samples were drawn before and after the treatment period of six-month. They were measured by ELISA method. The data were analyzed by SPSS software ver.16.0 using descriptive statistics and Paired Sample t test.

Results

The mean age of the children was 6.27 ± 2.25 years. There was a significant difference between the studied biomarkers before and after treatment ($P>0.05$) and the serum level of Immunoglobulin E, Eosinophil, Periostin and Eosinophil Cationic Protein was reduced after receiving the treatment.

Conclusion

The biomarkers serum levels in the children was reduced after the end of the treatment period. Thus, in this study, the role of selective biomarkers in asthma management was confirmed. The physicians could decide about the stop or continuation of the treatment by measuring their serum levels.

Key Words: Asthma, Biomarkers, Children.

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1- INTRODUCTION

It is estimated that asthma which has affected about 300 million people all over the world will be increased to 400 million people in 2025 (1). In addition, asthma is the most common chronic disease in the children which its incidence is estimated 5-10 percent (2, 3). In this disorder, the airways chronic inflammation results in limitations of extensive and varied reversible airflow, which cause various symptoms (2, 4). Genetic and environmental factors influence these disorders which their mechanisms have not been identified completely (5). These factors can influence the disease severity, patient response to treatment and symptoms such as recurrent wheezing, shortness of breath, and cough (6, 7). The mortality of asthma in the world is 0-0.7 in 100 children which can be prevented (2).

On time diagnosis, management of the symptoms and proper clinical decision are main factors in preventing the unpleasant consequences of disease (3). Diagnosis, determination of diseases severity and patients responses to treatment are basically done according to the patient history, physical exams and some para-clinical measures including spirometry (8, 9). In other words, decision making about the diseases is done traditionally based on the airway functions, drugs and the patient symptoms which most of them are nonspecific and subjective.

Also, many of the diagnostic tests do not show the main pathology clearly. Thus, the physicians cannot predict the nature of disorder and level of lower airway inflammation well and it is possible that their assessments show different results in the patients. The other challenge is that some of the children are young and they are not able to collaborate in the lung function tests (10). According to the inflammatory nature of asthma, many immune system cells play a role in this disorder (2). Investigation of these cells and their products known as inflammatory biomarkers might be helpful in diagnosis and assessment of diseases severity and response to treatment (11, 12). An ideal biomarker should be influential in diagnosis and determination of the diseases severity and response to treatment and can be non-invasive, low cost and measurable (11). In this regard, due to this fact that asthma is an allergic disease as type I sensitivity dispersion and considering the role of Eosinophil and Immunoglobulin E (IgE) in increase of this sensitivity and numerous reports on enhancement of Eosinophil and IgE in the patients with asthma (13-15); these factors can be specified as asthma biomarkers (13, 15). Since Eosinophil are increased weeks before severity of the disease, so the common examination methods cannot be as accurate as the para-clinical values for measuring inflammation and function of the airway (10).

Recently, Eosinophil is the most available biomarker for management of asthma (16). In addition, in recent years, the studies have shown that the proteins derived from Eosinophil can be considered as indicators evaluating asthma which Eosinophil Cationic Protein (ECP) has been investigated mostly among them (17). This protein is one of the most important cation granular proteins secreted from activated Eosinophil and nowadays, it is one of the important biomarkers used for evaluation of Eosinophil activity in asthma (17). Other biomarker is Periostin as a secreted extracellular matrix protein that plays a key role in increase of inflammation in allergic diseases. This protein leads to increase of Eosinophil in the tissue during inflammation and allergic processes. In this way, it seems that Periostin is associated with the epithelial fibroses process in asthma (11). Various studies have shown that the levels of Eosinophil and IgE in the patients with asthma are higher than control groups (16, 18, 19).

Some other ones did not show the relationship between the IgE level and severity of asthma (20, 21). Also, it seems that secretion of IgE antibody is mostly controlled by genetic factors, so this biomarker is not a proper predictor of asthma (18). The findings of some studies on the levels of eosinophil cationic protein (ECP) and Periostin showed that the levels of the biomarker ECP (22, 23), and Periostin (24-26) along with other inflammatory factors are significantly higher in the patients with asthma and they have statistical significant relationship with the severity of disease. However, one of studies doesn't show the significant difference in Periostin levels between the asthma group and control group (27). Therefore the necessity of conducting more studies for confirmation of the findings related to Periostin biomarker has been referred (28). Considering the current evidence, there are some conflicting reports on the mentioned biomarkers (7, 10, 11, 17) that depicts necessity of more investigations.

Also, most of the conducted studies in this regard have compared the serum level of these biomarkers in both asthma and control groups and a few research has investigated the level of biomarkers after treatment of asthma (16, 18, 19, 22, 24). In addition a few studies have done on children. While, the children are less able to express the symptoms and conducting clinical examination is difficult for them. The incidence of asthma in Tehran among children 7-18 years has been reported one hundred percent that reveals the importance of this disease in Iran (29). Considering all the mentioned issues and this fact that the children asthma is mostly allergic type, examining the immune system factors and prediction of the disease stage in this age group seems essential. So measuring the levels of IgE, Eosinophil, Periostin, and eosinophil cationic protein (ECP) in blood of children with asthma can be helpful in evaluation of response to treatment. Thus, in order to investigate the role of biomarkers, this study examines the changes of serum levels of IgE, eosinophils, Priestin and ECP which may occur as a result of treatment in children with asthma.

2- MATERIALS AND METHODS

This study with before and after design, was conducted in 2017 and children who referring to the respiratory clinic of Tabriz University of Medical Sciences were examined. For determining the sample size, the mean and standard deviation of pre-treatment (25.78±10.38), and post-treatment (20.86±5.17) of ECP in Guo's study were used (30). Considering the power of 95% and confidence interval of 95% in G power software Ver. 3.1.2, the sample size was estimated 46 children and considering 20% attrition rate, it calculated 56 children. The sampling was done using convenience method. The inclusion criteria were children at age 4 to 16 year with primary diagnosis of asthma. In order to receive the same interventions, children with moderate persistent asthma entered the study.

Criteria for moderate asthma have a mild airway obstruction, noticeable asthmatic symptoms during most to all days, nocturnal symptoms at least weekly, forced expiratory volume in one second (FEV_1), and forced expiratory volume to the forced vital capacity (FEV_1/FVC) measures are 60% to 80%, and reporting frequent slowed play and missed schooldays (3). Also, the children who suffer from another disorder in addition to asthma or who did not complete the treatment were excluded. This study approved by the Ethics Committee of Tabriz University of medical sciences (No. 493806 and ethics code: IR.TBZMED.REC.1396.381). Verbal and written consent was obtained from all parents of eligible children and they were

assured that all information would be kept confidential. The children demographic characterization and informations about their disease were documented in the questionnaire. For measuring the before-treatment biomarkers serum levels, peripheral venous blood was withdrawn. Then, the children were treated for six months under the drug therapy of moderate persistent asthma according to Kendig and Chernick's protocol (3). Therfore, all of the children received same intervention with medications of short-acting beta-agonist (SABA), inhaled corticosteroid (ICS), long-acting beta-agonist (LABA), leukotriene receptor antagonist (LTRA) and Methylxanthines.

Dosages of these medications arranged based on child's age. After six months treatment period, the blood sampling was done for second time for measuring the after-treatment biomarkers serum levels. The blood samples were collected in two tubes, one including EDTA anticoagulant (2 ml) and other without anticoagulant (3 ml). The tubes with EDAT were examined radially for complete blood count (CBC) for measuring the blood counts. The samples without anticoagulant were centrifuged with 3, 000 g for ten minutes and the serum samples were removed. The serum samples were divided into small vials (Ellicott) and transferred into freezer -70 degrees Celsius before conducting the test. Thus, the ECP, Periostin and IgE biomarkers were measured by Enzyme linked immunosorbent assay (ELISA) in the serum samples. It should be noted that the present study was single-blind. In this way, a researcher who measured the laboratory values was uninformed about the fact that the sent samples were related before or after the intervention. After collecting the data, the statistical analysis was done using software SPSS version 16. The descriptive statistics included frequency, percentage; mean and standard deviation (SD) were used for describing

the patients' characteristics and their serum levels. For comparison of the statistical difference between the pre-treatment and post-treatment groups, the inferential statistics were used that according to data normality distribution, the Paired Samples t Test was employed. For evaluating correlation between demographic data and serum levels, the independent t test and Pearson correlation coefficient were used. P-value less than 0.05 were considered significant statistically.

3- RESULTS

Of the 56 children with asthma, two children were excluded due to incomplete treatment period and the data analysis was done on 54 children. According to the results, 74% of the children were male and 26% was female. **Table.1** summarizes the mean and standard deviationof the children age. In order to investigate the role of biomarkers in the evaluation of the treatment and management of asthma, the results of the analysis showed that serum levels of Periostin, ECP, IgE and number and percentage of eosinophil decreased after six months of treatment. Based on the results of paired t-test, there was a significant difference between the mean biomarkers studied in beginning of the study and the six months later. The mean and standard deviation of serum biomarker levels before and after treatment and the t-test results are presented in **Table.2**.

Comparison of mean blood cells before and after treatment showed a significant difference in white blood cells and erythrocyte sedimentation rate as presented in **Table.3**. In addition to the main findings, regarding the relationship between the demographic variables and the biomarkers levels, result of independent t-test showed that there was no significant relationship between gender and serum biomarkers in pre-treatment and post-treatment measurements (P>0.05). Also,

based on Pearson test results, there was no significant relationship between age and serum biomarkers in pre-treatment and post-treatment measurements (P>0.05).

Table-1: The mean age of the studied children

Age (month)	Mean (SD)	Min	Max	Number
Male	76.85 (28.72)	28	154	40
Female	73.85 (22.81)	48	115	14
Total	75..33 (27.1)	28	15440	54

Table-2: The comparison of the biomarkers in asthmatic children before and after the treatment.

Biomarkers	Test	Mean	Std. deviation	t⁻test	P-value
ECP (pg/mL)	before	46.8033	41.08153	7.697	.000
	after	41.5822	38.04652		
POSTN (pg/mL)	before	56.8926	38.81358	3.968	.000
	after	48.9630	31.63296		
Eos (%)	before	5.1504	3.06827	2.817	.007
	after	4.0889	1.58587		
Eos Count(×1/μL)	before	579.7641	374.82635	5.355	.000
	after	315.7407	139.18024		
Serum total IgE (IU/ML)	before	341.362	198.235	6.221	.000
	after	138.875	101.312		

Statistical analyses were performed by paired-samples t-test; ECP: eosinophil cationic protein; POSTN: periostin; Eos: eosinophil; IgE: immunoglobulin.

Table-3: The comparison of the blood cell in asthmatic children before and after the treatment.

Blood Cells	Test	Mean	Std. deviation	t-test	P- value
Hb (g/dl)	before	13.1000	1.10778	1.208	.232
	after	13.0111	.93419		
PLT (×10³/μL)	before	316.41	57.93	2.011	.079
	after	304.74	43.71		
ESR (min)	before	9.0370	7.71415	2.480	.016
	after	6.5926	4.16870		
WBC (×1/μL)	before	11338.8889	4047.41924	6.995	.000
	after	8150.3704	2789.23061		

Statistical analyses were performed by paired-samples t-test; Hb: hemoglobin; ESR: erythrocyte sedimentation rate; PLT: platelet; WBC: white blood cell.

4- DISCUSSION

In this study, the levels of Periostin and ECP biomarkers and the number and percentage of eosinophil and IgE before and after treatment in the blood of children with asthma were investigated. Based on the findings, serum levels of Periostin decreased significantly after 6 months treatment. This finding is similar to the results of related studies. Izuhara et al. and Parulekar et al. showed that the serum levels of Periostin can show asthma prognosis and how to respond to treatment (26, 28). The study by Inoue et al. confirmed the higher serum levels of Periostin in children with asthma than in healthy individuals (24). It can be said that serum Periostin is animportant biomarker

for asthma due to two main reasons. First, this protein is easily released from the inflamed tissue into the bloodstream. Therefore, its serum level indicates the amount of its production from inflamed tissue and the extent of inflammation. Secondly, the serum levels of this protein are physiologically (normal) very low (about 50 ng / ml) (11). The Periostin sensitivity to the severity of illness is to the extent that Asano et al. in their study showed that the serum level of this protein in patients with asthma along with upper airway disorders such as rhinosinusitis and nasal polyps are significantly higher than in patients suffering from asthma without these disorders (26). In another study, James and colleagues showed that there was a significant correlation in Periostin in the patients with asthma and inflammatory factors and the lung activity, but there was no significant difference between the two groups of asthma and healthy control in Periostin level (27). This study was performed in patients aged 17-76 with rhinitis and sinusitis, which can lead to different results.

According to the results of the current study, there was a statistically significant difference between serum ECP levels. Pham et al. in their study showed that ECP level and eosinophil count, unlike other evaluated inflammatory factors, decreased in asthmatic patients after drug therapy, which could indicate the sensitivity of these factors to asthma treatment (31). Jiang and colleagues also stated that ECP level is related to the severity of the disease and ECP levels in asthmatic patients, besides other inflammatory factors, is significantly higher than healthy individuals (22). According to the results of a study by Niimi et al., the serum level of ECP in patients with asthma indicates the severity of inflammation of the respiratory tract and therefore may be related to the severity of the disease (23). Navratil also identified ECP as the best

predictor of asthma in management and treatment of this disease (32). Based on the findings of the current study and the results of other studies, it can be concluded that the ECP biomarker is more useful for assessing the severity of asthma and the response to treatment. A systematicstudy indicated that ECPbiomarker is also increased in other atopic diseases, but this biomarker is not suitable for diagnosis and due to the increase in ECP in airway inflammation, it is beneficial for evaluating the progress of treatment and control of the disease (17). According to the findings of the current study, the number and percentage of eosinophil and serum IgE levels had a significant relationship before and after asthma treatment. In two studies conducting on the children aged between 1 and 12 years old (19), and adults (18), the eosinophil and IgE values were compared in both healthy and patient groups. In the patient group, the eosinophil and IgE values were significantly higher than the healthy group.

But in other studies conducted on the individuals aged 1 to 80 years old (20), and 15 to 55 years old (21), there was no significant relationship between the severity of asthma and IgE levels. This difference can be due to the age range of the samples of these studies. Because the most common type of asthma in children is allergic (33) and IgE is more likely increased following allergic reactions (34). Patients with asthma experience clinical specifications and varying treatmentresponses, and control of the progression of treatment cannot be effective only by obtaining clinical history and subjective symptoms. Considering the findings of the present study and comparing them with existing evidences, determined the importance of biomarkers in the management of asthma. Due to the high accuracy of these biomarkers, the severity of the disease is recognizable. Accordingly, it is possible to estimate the

period of treatment and the condition of the disease in people who do not have any specific clinical symptoms or young children who are not able to express their conditions. Also it is possible to predict the disease progress and its consequences.

4-1. Limitations of the study

This research has only explored the mentioned biomarkers role. Thus, it is recommended to conduct other studies by the aim of examining the serum level of other factors such as interleukins and their relationship with these research biomarkers. Also, due to the fact that the intervention in the study was asthma treatment, there was no possibility that some of the children be deprived of treatment and placed in the control group. In this regard, this study was conducted without control group.

5- CONCLUSION

The research results showed the ECP, Eosinophil, Periostin and IgE serum levels were reduced in the children with asthma at the end of the treatment period. Thus, in this study, the role of selective biomarkers in management of the children with asthma was confirmed. The physicians could decide on time regarding the stop or continuation of the asthma treatment by measuring their serum levels. Therefore, it can avoid prescribing of more drugs in the case that there is no need for continuation of the treatment and reduce the patient and the society costs. Also, the physicians are able to change the disease symptoms from the subjective state to objective and measurable state reliably by measuring these biomarkers serum level.

6- CONFLICT OF INTEREST: None.

7- ACKNOWLEDGMENT

8- REFERENCES

1. Ober C, Yao TC. The genetics of asthma and allergic disease: a 21st century perspective. Immunological reviews. 2011;242(1):10-30.

2. Asher I, Pearce N. Global burden of asthma among children. The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2014;18(11):1269-78.

3. Kendig EL, Wilmott RW, Chernick V. Kendig and Chernick's Disorders of the Respiratory Tract in Children. 8th ed: Elsevier/Saunders; 2012. 1141 p.

4. Papaiwannou A, Zarogoulidis P, Porpodis K, Spyratos D, Kioumis I, Pitsiou G, et al. Asthma-chronic obstructive pulmonary disease overlap syndrome (ACOS): current literature review. Journal of Thoracic Disease. 2014;6(Suppl 1):S146-51.

5. Martinez FD. Genes, environments, development and asthma: a reappraisal. The European respiratory journal. 2007;29(1):179-84.

6. Choudhry S, Seibold MA, Borrell LN, Tang H, Serebrisky D, Chapela R, et al. Dissecting Complex Diseases in Complex Populations: Asthma in Latino Americans. Proceedings of the American Thoracic Society. 2007;4(3):226-33.

7. Pavord ID, Afzalnia S, Menzies-Gow A, Heaney LG. The current and future role of biomarkers in type 2 cytokine-mediated asthma management. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2017;47(2):148-60.

8. Lemanske RF, Busse WW. Asthma: Clinical Expression and Molecular Mechanisms. The Journal of allergy and clinical immunology. 2010;125(2 Suppl 2):S95-102.

9. Smith AD, Cowan JO, Filsell S, McLachlan C, Monti-Sheehan G, Jackson P, et al. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. American journal of

respiratory and critical care medicine. 2004;169(4):473-8.

10. Kim CK. Eosinophil-derived neurotoxin: a novel biomarker for diagnosis and monitoring of asthma. Korean journal of pediatrics. 2013;56(1):8-12.

11. Chiappori A, De Ferrari L, Folli C, Mauri P, Riccio AM, Canonica GW. Biomarkers and severe asthma: a critical appraisal. Clinical and molecular allergy : CMA. 2015;13:20.

12. Matsumoto H. Serum periostin: a novel biomarker for asthma management. Allergology international : official journal of the Japanese Society of Allergology. 2014;63(2):153-60.

13. Kono M, Mochizuki H, Arakawa H, Kato M, Tokuyama K, Morikawa A. Age-dependent relationship between bronchial hyperresponsiveness to methacholine and total serum IgE level in asthmatic children. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2001;87(1):33-8.

14. Tsilogianni Z, Hillas G, Bakakos P, Aggelakis L, Konstantellou E, Papaioannou AI, et al. Sputum interleukin-13 as a biomarker for the evaluation of asthma control. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 2016;46(11):1498.

15. Silvestri M, Oddera S, Spallarossa D, Frangova Yourukova V, Rossi GA. In childhood asthma the degree of allergen-induced T-lymphocyte proliferation is related to serum IgE levels and to blood eosinophilia. Annals of allergy, asthma & immunology : official publication of the American College of Allergy, Asthma, & Immunology. 2000;84(4):426-32.

16. Gibson PG. Variability of blood eosinophils as a biomarker in asthma and COPD. Respirology (Carlton, Vic). 2018;23(1):12-3.

17. Koh GC, Shek LP, Goh DY, Van Bever H, Koh DS. Eosinophil cationic protein: is it useful in asthma? A systematic review. Respiratory medicine. 2007;101(4):696-705.

18. Akbari Aliabad S, Nabavizadeh S, Mossavizadeh SA, Hadinia A. Correlation between Serum Levels of Total IgE Antibodyand Percent of Eosinophil Cellsin Patients with Asthma Referred to Yasuj Mofatteh Clinic in 2013. YUMSJ. 2014;18(10):797-804.

19. Hashemzadeh A, Heydarian F, Hashemzadeh S. Evaluation of total IgE and eosinophils in children with asthma. Horizon of Medical Sciences. 2005;10(4):23-7.

20. Roshanizadeh Z, Ghadiri A, Khodadadi A, Ghandil P, Parsanahad M, Tavakol H, et al. A correlation between on clinical finding and IgE measurement on asthma patients Arab ethnicity and Bakhtiari in Khuzestan province. Jundishapur Scientific Medical Journal. 2016;14(6):642-52.

21. Saba MA, Akbari H, Banihashemian SM, Jazayeri H, Talaei SA, Banihashemian SS, et al. Relationship between serum levels of IL-4 and IgE with disease severity in allergic asthma. Feyz Journal of Kashan University of Medical Sciences. 2013;17(4):366-72.

22. Jiang XG, Yang XD, Lv Z, Zhuang PH. Elevated serum levels of TNF-alpha, IL-8, and ECP can be involved in the development and progression of bronchial asthma. The Journal of asthma : official journal of the Association for the Care of Asthma. 2018;55(2):111-8.

23. Niimi A, Amitani R, Suzuki K, Tanaka E, Murayama T, Kuze F. Serum eosinophil cationic protein as a marker of eosinophilic inflammation in asthma. Clinical and experimental allergy : journal of the British Society for Allergy and Clinical Immunology. 1998;28(2):233-40.

24. Inoue T, Akashi K, Watanabe M, Ikeda Y, Ashizuka S, Motoki T, et al. Periostin as a biomarker for the diagnosis of pediatric asthma. Pediatric allergy and immunology : official publication of the European Society of Pediatric Allergy and Immunology. 2016;27(5):521-6.

25. Asano T, Kanemitsu Y, Takemura M, Yokota M, Fukumitsu K, Takeda N, et al. Serum Periostin as a Biomarker for Comorbid Chronic Rhinosinusitis in Patients with

Asthma. Annals of the American Thoracic Society. 2017;14(5):667-75.

26. Izuhara K, Ohta S, Ono J. Using Periostin as a Biomarker in the Treatment of Asthma. Allergy, asthma & immunology research. 2016;8(6):491-8.

27. James A, Janson C, Malinovschi A, Holweg C, Alving K, Ono J, et al. Serum periostin relates to type-2 inflammation and lung function in asthma: Data from the large population-based cohort Swedish GA(2)LEN. Allergy. 2017;72(11):1753-60.

28. Parulekar AD, Atik MA, Hanania NA. Periostin, a novel biomarker of TH2-driven asthma. Current opinion in pulmonary medicine. 2014;20(1):60-5.

29. Mirsaeid Ghazi B, Sharifi SH, Goodarzipoor K, Aghamohammadi A, Atarod L, Rezaei N, et al. The Prevalence of Asthma among the Students (7-18 Years Old) in Tehran during 2002-2003. Iranian journal of allergy, asthma, and immunology. 2004;3(2):89-92.

30. Guo CL, Sun XM, Wang XW, Guo Q. Serum Eosinophil Cationic Protein Is a Useful Marker for Assessing the Efficacy of Inhaled Corticosteroid Therapy in Children with Bronchial Asthma. The Tohoku journal of experimental medicine. 2017;242(4):263-71.

31. Pham TH, Damera G, Newbold P, Ranade K. Reductions in eosinophil biomarkers by benralizumab in patients with asthma. Respiratory medicine. 2016;111:21-9.

32. Navratil M, Plavec D, Erceg D, Bulat Lokas S, Zivkovic J, Turkalj M. Urates in exhaled breath condensate as a biomarker of control in childhood asthma. The Journal of asthma : official journal of the Association for the Care of Asthma. 2015;52(5):437-46.

33. Larsen GL. Differences between adult and childhood asthma. The Journal of allergy and clinical immunology. 2000;106(3 Suppl):S153-7.

34. Platts-Mills TA. The role of immunoglobulin E in allergy and asthma. American journal of respiratory and critical care medicine. 2001;164(8 Pt 2):S1-5.