MEDIA MEDIKA INDONESIANA

Effects of BCG Vaccination Prior to Specific Immunotherapy on IL-4/IFN-γ Ratio and AR Total Symptom Score in Persistent Allergic Rhinitis

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ABSTRAK

Background: *Allergic disease including allergic rhinitis (AR) is dominated by Th_2 responses with increased production of IL-4 and inhibition of IFN-γ production. BCG vaccination induced IL-12 production which promotes Th_0 to Th_1 shift and stimulates IFN-γ production. The aim of this study is to determine whether BCG vaccination prior specific immunotherapy (SIT) could increase initial phase of SIT responses measured by decreasing IL-4/IFN-γ ratio's and AR total symptom score (TSS).*

Method: *This study was carried out by a randomized controlled trial. A total of 84 patients with moderate to severe persistent AR were randomized and assigned into 4 treatment groups (BCG solvent, BCG vaccination, SIT, and BCG vaccination prior SIT). A subcutaneous SIT was given using mixed house dust mite extract (D pteronyssinus and D farinae, 50/50), while BCG vaccination was given by intracutaneous injection. Clinical symptoms and concentrations of IL-4 and IFN-γ were measured by PHA stimulated fresh whole blood culture, before and after 8 weeks of SIT.*

Result: *There were no significance difference between the concentrations of IL-4 and IFN-γ, and their ratio in all four groups. However, there is a significant lower IL-4/IFN-γ ratio in the group with pretreatment of BCG than SIT (p=0.024), while during the initial phase, AR symptoms in patients who received BCG prior SIT is significantly lower than SIT (p=0.027).*

Conclusion: *This study demonstrates that BCG vaccination prior to SIT treatment could increase initial phase of SIT responses in persistent allergic rhinitis without additional adverse effects.*

Keywords: *BCG vaccination, specific immunotherapy, allergic rhinitis*

ABSTRACT

Efek vaksinasi BCG pre imunoterapi spesifik terhadap rasio IL-4/IFN-γ dan skor gejala total rinitis pada rinitis alergi persisten

Latar belakang: *Pada penyakit alergi, termasuk rinitis alergi (RA) didominasi oleh respon Th_2 yang meningkatkan produksi IL-4 yang kemudian menghambat produksi IFN-γ. Vaksinasi BCG memicu produksi IL-12 yang menyebabkan pergeseran Th_0 ke Th_1 dan menginduksi produksi IFN-γ. Penelitian ini bertujuan untuk membuktikan bahwa vaksinasi BCG pre imunoterapi spesifik (IT) dapat meningkatkan respon IT spesifik selama fase inisial dengan mengukur rasio IL-4/IFN-γ dan skor gejala total rinitis alergi.*

Metode: *Sebanyak 84 pasien RA persisten sedang–berat yang memenuhi kriteria penelitian dilakukan randomisasi ke dalam 4 kelompok pengobatan (pelarut BCG sebagai kontrol, vaksinasi BCG, IT dan vaksinasi BCG pre IT). Imunoterapi spesifik disuntikkan sub kutan 2 kali seminggu selama 8 minggu dengan ekstrak campuran (D pteronyssinus dan D farinae, 50/50). Vaksinasi BCG diberikan secara intra kutan. Konsentrasi IL-4 dan IFN-γ diukur dari kultur darah segar yang distimulasi phytohemaglutinin (PHA). Rasio IL-4/IFN-γ dan skor gejala total RA dianalisis sebelum dan setelah IT spesifik 8 minggu.*

Hasil: *Tidak terdapat perbedaan bermakna antara konsentrasi IL-4 dan IFN-γ serta rasionya sebelum dan sesudah terapi pada semua kelompok pengobatan, tetapi rasio IL-4/IFN-γ pada kelompok BCG pre IT pada akhir fase inisial IT lebih rendah dibanding kelompok IT (p=0,024). Selama fase inisial IT, skor total gejala RA pada kelompok BCG pre IT juga lebih rendah secara bermakna dibanding kelompok IT (p=0,027).*

Simpulan: *Penelitian ini menunjukkan bahwa vaksinasi BCG pre IT meningkatkan respon fase inisial IT spesifik pada penderita rhinitis alergi persisten tanpa efek samping yang berarti.*

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INTRODUCTION

Allergic disease is dominated by Th_2 responses with increased production of IL-4 resulting in inhibition of IFN-γ production and induction of IgE production.[1,2] Allergic rhinitis (AR) may interfere the health status, impair quality of life and need a high cost of treatment. AR prevalence in USA was 14.2%, while in Semarang, Indonesia, the prevalence among 13-14 year old students was 18.6%.[3,4]

Specific immunotherapy (SIT) is an effective treatment for AR and has a long residual effects after discontinuation.[5] The effects of SIT consist of the shift of Th_2 to Th_1 cytokine production, decrease of the specific IgE and nasal mucosa eosinophils and increase of IgG_4 resulting in the decrease of AR symptoms.[6] It is recommended that patients with a moderate to severe persistent AR and are resistant to pharmacologic treatment need a specific immunotherapy.[7]

For the last decades, atopic diseases have been increased in the developed countries. The increase might be due to environmental changes, diet factors, the way of life and vaccination programs. There was an inverse association between tuberculin positive response and the low prevalence of atopic diseases in Japan.[8] BCG vaccination decreases an atopic disease since BCG vaccine is a Th_1 inducer for the production of endogenous IL-12 cytokine, thus in ducing the IFN-γ production. IL-12 cytokine also stimulates Th_1 and NK cells to produce IFN-γ.[9] An intranasal BCG infection showed a suppressive effect on eosinophils accumulation within the lung, which is the most pronounced suppressive effect obtained when BCG is given one week before the allergen challenge.[10] The nose and the lung are components of the respiratory tract, however, the effect of BCG in the nasal mucous membrane has not been determined. To test this hypothesis, we have conducted a study to determine production of IL-4 and IFN-γ, IL-4/IFN-γ ratio, and total symptoms score (TSS) in AR patients given BCG vaccination prior to specific immunotherapy compared to the end of initial phase of specific immunotherapy (SIT).

METHOD

This is a randomized controlled study. Patients with moderate to severe persistent AR, aged from 15-50 years old with a positive (+++) skin prick test results to house dust mite allergens, either *dermatophagoides pteronyssinus* or *dermatophagoides farinae*, were enrolled into the study.

The patients were excluded when taken antihistamine and anti-inflammatory drugs within 72 hours, systemic or topical corticosteroid within 2 weeks or have depot corticosteroid within 8 weeks. Patients who were pregnant or lactating, presence of sinusitis with or without nasal polyps, history of receiving SIT, severe allergic manifestations, cardiac abnormality, patients who took β-blocker or antihypertensive medication, pulmonary tuberculosis or Mantoux test more than 10 mm diameter were excluded from this study. All patients gave their informed consent and the study was approved by the hospital/faculty ethical committee.

Eligible patients were randomly assigned into 4 groups. First group received intracutaneous BCG solvent (Group I, the control group), the second group received intracutaneous BCG vaccination (Group II), the third group received a specific immunotherapy (Group III) and the forth group received BCG vaccination prior to the specific immunotherapy (Group IV). Mantoux test was done in the patients selection using PPD RT 23. Patients who have lost of follow-up for more than 4 weeks of treatment were considered as the drop-out cases.

The dose of BCG solvent was 0.1 ml and the same volume for BCG vaccine (2×10^5 CFU, Paris strain No. 1172 P2). Initial phase treatment of SIT comprised *dermatophagoides* mix allergens (*dermatophagoides pteronyssinus* and *dermatophagoides farinae*, 50/50*)* extract allergen (ALK-Abello^R) with varying concentrations of 1, 10, 100 and 1,000 STU/ml, respectively. Subcutaneous injection was given twice a week for initial dose phase (according to the manufacturers' instruction) for up to 8 weeks.

IL-4/IFN-γ ratio and AR total symptoms score (TSS) were determined in all study patients. IL-4 and IFN-γ were measured using phytohemaglutinin (PHA) stimulated fresh whole blood culture with compact human IL-4 and human IFN-γ ELISA Kit (Pelikine^R) at the Biotechnology Laboratory, the Faculty of Medicine, Diponegoro University. Whole blood culture was used to anticipate a very low level of cytokines in the serum. The advantage of using a whole blood culture were a short incubation time, simple and similar to the *in vivo* conditions.[11] PHA stimulation was chosen because at the preliminary study gave the best result compared to LPS and mite antigen. The sensitivity of the test was 0.2 pg/ml for IL-4 and 1 pg/ml for IFN-γ.

The major allergic rhinitis symptoms included sneezing, rhinorhea, nasal obstruction and nasal itching which were measured subjectively using a 4-point scale by diary card. Scale 0 (zero) is when there was no allergic rhinitis symptoms, scale 1 (mild) when allergic rhinitis symptoms do not disturb patient's activity, scale 2 (moderate) when allergic rhinitis symptoms disturb patient's activity/sleep and scale 3 (severe) when allergic rhinitis symptoms disturbed the patient's activity/sleep.[12]

The sample size was calculated based on 50% of reduced TSS score in BCG+SIT as compared to SIT along group, 0.80 power of the study and significant level of 0.05. To compare patient's characteristic between treatment group were analyzed by Chi square test for nominal data, Kruskal Wallis test for normally distributed data and using analysis of variants for not normally distributed data. Since the data of IL-4, IFN-γ cytokines and their ratio were not normally distributed, log transformation was used for analyzing these data.

The difference between cytokines (IL-4 and IFN-γ) levels before and after treatment were analyzed by paired t-test. The difference of IL-4/IFN-γ ratio and allergic rhinitis TTS before and after treatment were determined by Wilcoxon Signed Ranks tests. At the end of treatment, the comparison of IL-4/IFN-γ ratio and allergic rhinitis TTS between each treatment group were determined by Mann Whitney U test.

Since AR TSS was evaluated everyday during 8 weeks, the Area Under the Curve (AUC) NCSS 2000–PASS200 program was used to compare the AUC during SIT and BCG prior SIT groups. MANOVA test was used to determine whether sex, age, duration of illness and allergic family history influenced the treatment results.

RESULTS

Patients sampling were done during July 2004 to December 2005. A total of 84 patients have been recruited in this study and 73 (86.9%) patients have completed the study. However, analysis of the data was based on 55 cases who had a complete set of results of IL-4 and IFN-γ measurements, and symptom score records before and after the treatment. It was found that sex, age, allergic family history, duration of AR, IFN-γ level and IL-4/IFN-γ ratio, AR total symptom score before treatment were comparable between groups, except IL-4 cytokine (Table 1).

There were changes in IL-4 and IFN-γ production during the study, however, there was no significant difference between IL-4, IFN-γ and their ratio before and after treatment in all groups of treatment (Table 2). At the end of the study, however, IL-4/IFN-γ ratio decreased only in BCG prior SIT group and it was significantly lower than IL-4/IFN-γ ratio in SIT group (Table 3).

At the end of the study, the significant difference of AR TSS was found between: a) control (group I) and BCG groups (group II) (p=0.034), b) control (group I) and SIT groups (group III) (p=0.02), c) control (group I) and BCG prior SIT groups (group IV) (p=0.009) and BCG (II) and SIT groups (group III) (p=0.049). Allergic rhinitis TSS between SIT and BCG prior SIT groups was not significant different, however, during the period of study, using AUC analysis, the result showed that the AUC during SIT was significantly larger than the AUC during BCG prior SIT (p=0.027), and the difference was came from the nasal obstruction symptoms (p=0.005) (Fig. 1a, 1b, 1c and 1d). From the MANOVA test, it showed that age (p=0.167), sex (p=0.711) duration of rhinitis (p=0.190) and allergic family history (p=0.9) do not influence the treatment results.

Table 1. Sex, age, duration of illness, family history of allergy, median TSS, median nasal mucosa eosinophils, IL-4 and IFN-γ cytokines and their ratio before treatment

Groups	I Control (n=21)	II BCG (n=21)	III SIT (n=21)	IV BCG + SIT (n=21)	*p*-value
Sex					
Male	10	7	8	9	0.80*
Female	11	14	13	12	
Age (years)	25	26.9	31.58	28	0.429**
Family history of allergy					
Positive	15	8	12	11	0.188*
Negative	6	13	9	10	
Duration of illness (months)	45.5	51	89	59	0.297**
Median AR TSS	11	10	9	10	0.321**
Median nasal mucosa eosinophil count	44	23	9	12	0.123**
Median IL-4 (pg/ml)	14.5	16.21	61.52	40.37	0.017***
Median IFN-γ (pg/ml)	923.21	1,347.72	2,375.75	2,374.65	0.270***
IL-4/IFN-γ ratio	0.015	0.012	0.026	0.017	0.080**

* Chi-square test, ** Kruskal Wallis test, *** Analysis of variants

Table 2. Cytokine levels and their ratio before and after treatment (log transformation data)

	Control n=10	BCG n=14	SIT n=17	BCG+SIT n=14
1. IL-4				
Before	14.15 ↑	16.21 ↑	61.52 ↓	40.37 ↓
After	19.44	30.63	56.88	37.70
p-value	0.434	0.109	0.835	0.917
2. IFN-γ				
Before	923.21 ↑	1,347.72 ↓	2,375.75 ↓	2,374.65 ↑
After	1,170.31	1,159.04	1,585.26	2,659.50
p-value	0.780	0.728	0.256	0.821
3. IL-4/IFN-γ ratio				
Before	0.015 ↑	0.012 ↑	0.026 ↑	0.017 ↓
After	0.017	0.026	**0.036**	**0.014**
p-value	0.878	0.124	0.906	0.826

p-value (Willcoxon Signed Ranks test)

Table 3. Comparison of IL-4/IFN-γ ratio between groups at the end of the treatment (p value of Mann Whitney test)

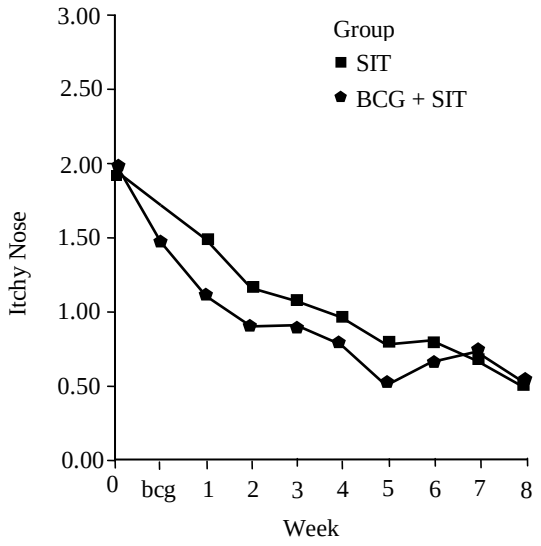
Group	BCG	IT	BCG + IT
Control	*p*=0.048	*p*=0.015	*p*=0.292
BCG		*p*=0.355	*p*=0.082
IT			***p*=0.024**

Adverse effects of the treatments such as headache, itchy at the site of SIT injection and malaise were reported by some patients in each group, however, there was no significant difference between groups of treatment. Wheal and pustule formations at the site of injection were found in all patients who received BCG vaccination, however, it did not make patients discontinued their participation to the study.

DISCUSSION

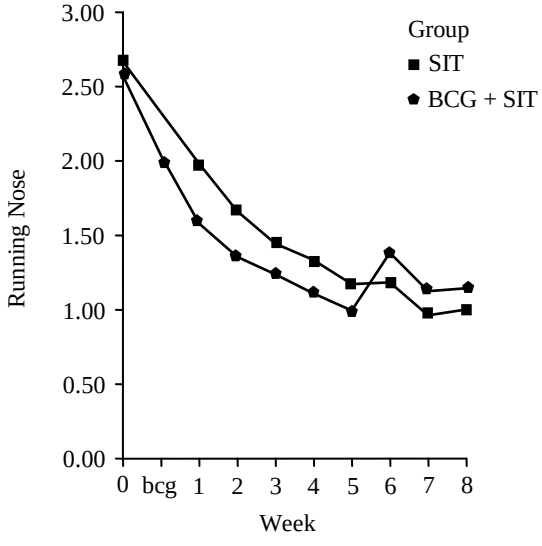
At the end of the study, either IL-4 (increased 37.38%) or IFN-γ (increased 26.76%) production were increase in control group (group I), as well as for the IL-4/IFN-γ ratio. It means that BCG solvent injection did not change the domination of Th_2 response in allergic rhinitis patients. In the BCG vaccination group (group II), IL-4/IFN-γ ratio has also increased at the end of the study which was due to the increase of IL-4 (88%) and decrease of IFN-γ (14%) production. It was reported that BCG vaccination could induce similar immune response to CD_4^+ T cells and T-CD_8^+ hsp-65 reactive cells in the spleen. After BCG vaccination, most stimulated cells were $CD4_{l0}$ which produced IL-4 besides IFN-γ.[21] Furthermore, the highest T cell response to PPD was found 2 weeks after BCG vaccination and it was parallel with the maximum IFN-γ production, which has decreased after 8 weeks of BCG infection.[10]

In the SIT group (group III), IL-4/IFN-γ ratio during escalating dose phase has increased (38.46%), and this finding is consistent with the previous study.[13] The increase of IL-4/IFN-γ ratio in SIT group is caused by relatively higher production of IL-4 when compared to IFN-γ production. This phenomenon might be due to a low dose of allergen injection during escalating dose phase, in which dendritic cells involved in immunologic reactions are type-2 (DC-2) which would induce transcription factor for IL-4 production.[14] It has been reported that in atopic patients the number of type-2 dendritic cells (DC-2) increased significantly compared to non-atopic person.[15] In fact, at the end of this study (after 8 weeks of treatment) in SIT group, either IL-4 (decreased 7.54%) or IFN-γ (decreased 33.27%) has decreased and this might be caused by the role of regulatory T cells, since it was reported in semi-rush SIT that T cells produced IL-10 and TGF-β. Both cytokines are produced by $CD4^+.CD25^+$ T-cells and could be detected on day 7 of semi-rush SIT.[16] Regulatory T (T_{reg}) cells produced IL-10 and TGF-β induced by dendritic cells during allergen injection and



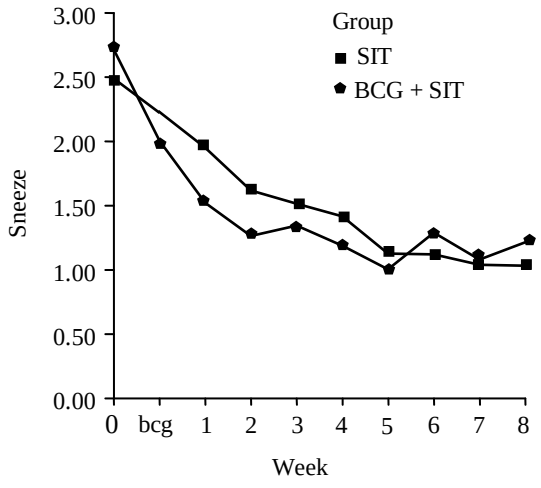
Mann-Whitney U test, AUC; *p*=0,112

Figure 1a. Median score of itchy nose



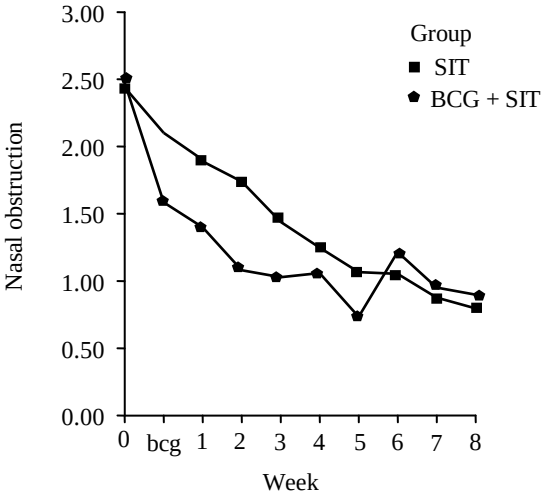
Mann Whitney U test, AUC; *p*=0,09

Figure 1b. Median score of rhinorhea



Mann Whitney U test, AUC; *p*=0,138

Figure 1c. Median score of sneezing



Mann Whitney U test, AUC; *p*=0,005

Figure 1d. Median score of nasal obstruction

both cytokines suppressed the production of type-2 cytokines (IL-4, IL-5) and type-1 cytokines (IFN-γ).[17]

At the end of the study, IL-4/IFN-γ ratio in group IV (BCG vaccination prior SIT) has decreased and it was significantly lower than in SIT group (*p*=0.024), due to increased IFN-γ production and reduced IL-4 production. This might be caused by intracellular infection of macrophages by BCG vaccine which then induced endogen IL-12 production.[18] IL-12 is an important cytokine micro-environment for Th_0 to Th_1 polarization and stimulates IFN-γ production by NK cells and Th_1 cells. Furthermore, the increase of IFN-γ production would suppress IL-4 production by Th_2 cells.[19,20]

It has been reported that the median of IL-4/IFN-γ ratio in normal person was 1:117 (0.000854) but in AR patients was 1:4 (0.25) (p=0.00067).[12] In allergic rhinitis, a treatment was considered successful in the immunological aspect when there is a decrease of IL-4/IFN-γ ratio or a shift of cytokines production from Th_2 to Th_1 response. Therefore, it can be concluded that BCG vaccination prior SIT plays an important role as an immunological inducer for cytokine type-1 response in AR patients during the initial phase dose of SIT. This result is consistent with the decrease of allergic rhinitis TSS, where the AUC of AR TSS during SIT was significantly larger than the AUC of AR TSS during BCG prior SIT where the difference was mainly

contributed by the improvements of nasal obstruction symptom. This immunological effect was not influenced by sex, age, duration of rhinitis and allergic family history of patients.

CONCLUSION

This study demonstrates that BCG vaccination prior SIT plays an important role as an immunological inducer for cytokine type-1 response in AR patients during the initial phase dose of SIT. It will result in decrease of IL-4/IFN-γ ratio and improvement of AR TSS without additional adverse effects.

ACKNOWLEDGMENTS

I thank dr. Kis Jamiatun, MSc and all laboratory member for their valuable help throughout the study.

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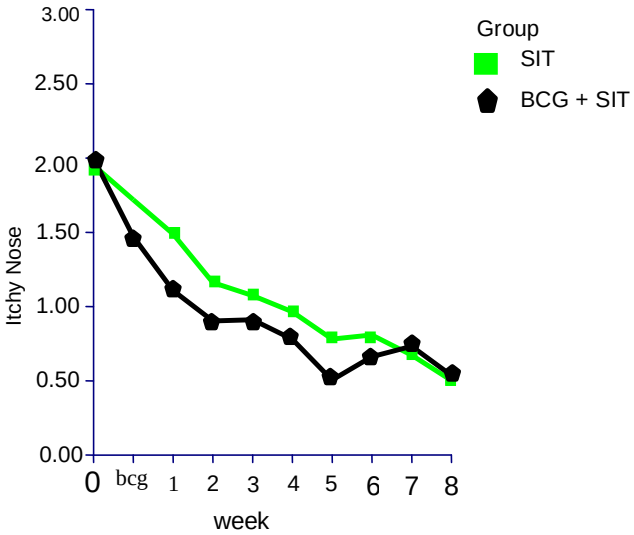
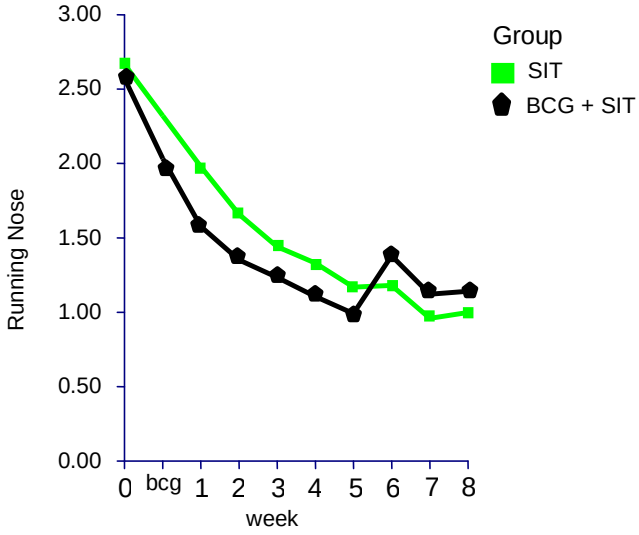
Sinopsis

Vaksinasi BCG pre IT meningkatkan respon fase inisial IT spesifik pada rinitis alergi persisten tanpa efek samping yang berarti..



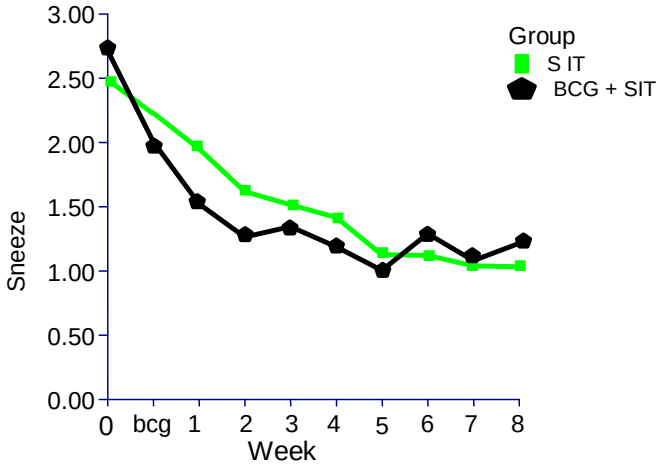
Mann- Whitney U test ; AUC; p= 0,112

Figure 1a. Median score of itchy nose



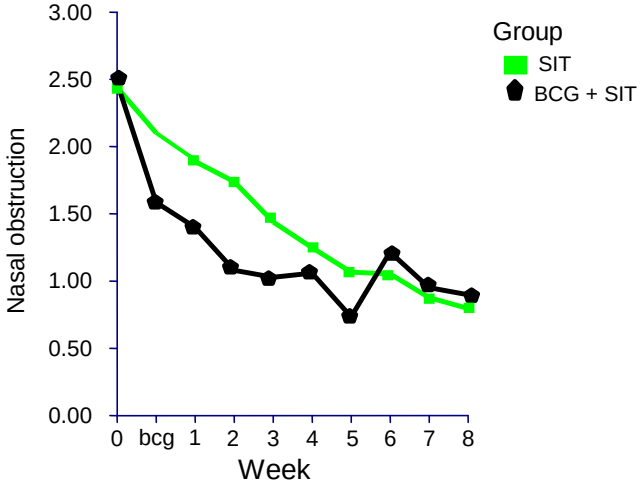
Mann Whitney U test ; AUC ; p = 0,09

Figure 1b. Median score of rhinorhea



Mann Whitney U test s AUC ; p= 0,138

Figure 1c. Median score of sneezing



Mann Whitney U test AUC ; p= 0,005

Figure 1d. Median score of nasal obstruction