

Effects of a short course of inhaled corticosteroids in noneosinophilic asthmatic subjects

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BACKGROUND: Noneosinophilic asthma has been regarded as a distinct phenotype characterized by a poor response to inhaled corticosteroids (ICS).

OBJECTIVE: To determine whether noneosinophilic, steroid-naïve asthmatic subjects show an improvement in asthma control, asthma symptoms and spirometry after four weeks of treatment with ICS, and whether they further benefit from the addition of a long-acting beta-2 agonists to ICS.

METHODS: A randomized, double-blind, placebo-controlled, multicentre study comparing the efficacy of placebo versus inhaled fluticasone propionate 250 µg twice daily for four weeks in mildly uncontrolled, steroid-naïve asthmatic subjects with a sputum eosinophil count ≤2%. This was followed by an open-label, four-week treatment period with fluticasone propionate 250 µg/salmeterol 50 µg, twice daily for all subjects.

RESULTS: After four weeks of double-blind treatment, there was a statistically significant and clinically relevant improvement in the mean (± SD) Asthma Control Questionnaire score in the ICS-treated group (n=6) (decrease of 1.0±0.5) compared with the placebo group (n=6) (decrease of 0.09±0.4) (P=0.008). Forced expiratory volume in 1 s declined in the placebo group (-0.2±0.2 L) and did not change in the ICS group (0.04±0.1 L) after four weeks of treatment (P=0.02). The open-label treatment with fluticasone propionate 250 µg/salmeterol 50 µg did not produce additional improvements in those who were previously treated for four weeks with inhaled fluticasone alone.

CONCLUSION: A clinically important and statistically significant response to ICS was observed in mildly uncontrolled noneosinophilic asthmatic subjects.

Key Words: Asthma; Asthma Control Questionnaire; Eosinophils; Sputum cell counts

Inhaled corticosteroids (ICS) are considered to be the cornerstone of asthma treatment according to international asthma guidelines (1). Their use is recommended as the first line of therapy in patients with mildly uncontrolled asthma (1,2). However, the response to ICS treatment does not appear to be uniform among asthmatic patients. Indeed, some patients do not seem to benefit from this treatment to the same extent as others. Factors such as smoking seem to alter the response to ICS (3); however, the main factor identified for the prediction of a poor response to ICS has been a low sputum eosinophil count. Indeed, noneosinophilic asthma has been regarded as a distinct phenotype characterized by a poor response to ICS (4,5). Several studies have reported limited improvement in respiratory function tests in noneosinophilic asthmatic subjects after ICS treatment compared with eosinophilic asthmatic subjects (4,5). We previously observed an improvement in forced expiratory volume in 1 s (FEV₁) and bronchial responsiveness (provocative concentration of methacholine causing a

Les effets d'un court traitement par corticothérapie par aérosol chez des sujets atteints d'asthme non éosinophile

HISTORIQUE : L'asthme non éosinophile est perçu comme un phénotype distinct, caractérisé par une mauvaise réponse à la corticothérapie par aérosol (CTA).

OBJECTIF : Déterminer si les sujets atteints d'asthme non éosinophile naïfs aux stéroïdes améliorent leur contrôle de l'asthme, leurs symptômes d'asthme et leur spirométrie après quatre semaines de traitement par CTA, et s'ils profitent davantage de l'ajout de bêta-2 agonistes à action prolongée à cette CTA.

MÉTHODOLOGIE : Une étude aléatoire multicentrique à double insu, contrôlée contre placebo, a permis de comparer l'efficacité d'un placebo par rapport à 250 µg de propionate de fluticasone par aérosol deux fois par jour pendant quatre semaines chez des sujets asthmatiques naïfs aux stéroïdes légèrement mal contrôlés et dont la numération des éosinophiles dans les expectorations était égale ou inférieure à 2 %. Cette posologie était suivie d'un traitement ouvert de quatre semaines de 250 µg de propionate de fluticasone et de 50 µg de salmétérol deux fois par jour chez tous les sujets.

RÉSULTATS : Après quatre semaines de traitement à double insu, les auteurs ont constaté une amélioration statistiquement significative et pertinente sur le plan clinique de l'indice moyen (± ÉT) du questionnaire de contrôle de l'asthme dans le groupe traité par CTA (n=6) (diminution de 1,0±0,5) par rapport au groupe placebo (n=6) (diminution de 0,09±0,4) (P=0,008). Le volume expiratoire maximal par seconde a diminué dans le groupe qui prenait un placebo (-0,2±0,2 L) et n'avait pas changé dans le groupe par CTA (0,04±0,1 L) après quatre semaines de traitement (P=0,02). Le traitement ouvert au moyen de 250 µg de propionate de fluticasone et de 50 µg de salmétérol n'a pas produit d'amélioration supplémentaire chez les patients qui avaient déjà été traités à la seule fluticasone par aérosol pendant quatre semaines.

CONCLUSION : On a observé une réponse à la CTA importante sur le plan clinique et statistiquement significative chez les sujets atteints d'asthme non éosinophile légèrement mal contrôlé.

20% fall in FEV₁ [PC₂₀] after one month of ICS treatment in noneosinophilic asthmatic subjects in an open-label uncontrolled study. However, there was a reasonable possibility that those improvements may have also occurred after treatment with placebo (6).

Sputum cellular analysis has increasingly been used in the management of asthma following confirmation that titrating ICS treatment in accordance with the percentage of sputum eosinophils resulted in a significant reduction in asthma exacerbations (7,8). Therefore, we reasoned that it was crucial to know whether subjects with a low eosinophil count (≤2%) could still benefit from ICS treatment. Accordingly, we designed a randomized, double-blind, placebo-controlled clinical trial to determine whether noneosinophilic, steroid-naïve asthmatic subjects show an improvement in their asthma symptoms and lung function after four weeks of treatment with ICS. We also assessed whether they further benefited from the addition of long-acting beta-2 agonists (LABAs) to ICS.

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METHODS

Study design

A randomized, double-blind, placebo-controlled, multicentre study was performed comparing the efficacy of inhaled fluticasone propionate 250 µg twice daily with placebo for four weeks in mildly uncontrolled, steroid-naïve asthmatic subjects with a sputum eosinophil count $\leq 2\%$. This was followed by an open-label, four-week treatment period with a combination of fluticasone propionate 250 µg/salmeterol 50 µg given by inhaler twice daily for all subjects. The study design and procedures performed at each visit are described in Figure 1. Sputum induction was performed on two occasions (visits 1 and 2) to ensure that the noneosinophilic phenotype was persistent. A third visit two weeks after the start of treatment (ie, visit 3) ensured that there was no deterioration of asthma in the study subjects. GlaxoSmithKline Inc provided the fluticasone propionate, placebo and fluticasone propionate/salmeterol combination, each as a dry powder (Diskus® inhaler), but had no further role in the design or conduct of the study, or in the collection, tabulation, analysis or interpretation of the data, or in any stage of manuscript preparation.

Subjects

Subjects were recruited from seven Canadian centres. Individuals who were between 18 and 70 years of age and had a diagnosis of asthma according to the Guidelines for the Diagnosis and Management of Asthma (9) were included. Included subjects had not been treated with ICS in the previous two months, had a $PC_{20} < 8$ mg/mL, or a reversibility of $FEV_1 > 12\%$ after bronchodilator if the methacholine inhalation challenge could not be performed. Subjects included in the study were required to have evidence of mildly uncontrolled asthma, as demonstrated by awakenings due to asthmatic symptoms at least once per week, or regular use of salbutamol on at least four occasions per week (excluding exercise prophylaxis) due to asthma symptoms. During a one-week interval before randomization, subjects had to have an Asthma Control Questionnaire (ACQ) score ≥ 2 on one visit with a variation of < 0.5 within a week, and a sputum eosinophil count $\leq 2\%$ on two separate visits. Subjects who had a smoking history of greater than 10 pack-years were excluded from the study. Subjects who had been hospitalized within the previous three months experienced current or recent (within the past month) cold or flu-like symptoms, had a history of near fatal asthma, or were taking inhaled corticosteroids, prednisone, LABAs, montelukast or theophylline within two months before entry into the study were also excluded.

The study was approved by the ethics committee of each participating centre, and each subject signed an informed consent form. The project was approved by the Ethics Committee of Montreal Sacré-Coeur Hospital (Montreal, Quebec) no: CE 2007-07-57. The present trial was registered at ClinicalTrials.gov: NCT00509197.

Procedures

1. Questionnaires. The clinical characteristics of the subjects were assessed by a standard questionnaire at the first visit. Asthma symptoms were assessed using the validated ACQ (10). Quality of life was assessed using the asthma quality of life questionnaire (AQLQ) specifically developed by Juniper et al (11).
2. Skin-prick tests with 12 common inhalant allergen extracts and a negative (diluent) and positive control (histamine 10 mg/mL) were performed by the modified prick method as described by Pepys (12). A result was documented as positive if the wheal was ≥ 2 mm in diameter compared with the negative control.
3. Spirometry. FEV_1 and forced vital capacity (FVC) were performed according to the standards of the American Thoracic Society (13) at clinic visits. Predicted values of FEV_1 and FVC were taken from Crapo (14).
4. Sputum induction and processing. Sputum induction with normal or hypertonic saline was adapted from a previously described technique (9). Sputum was processed as described by Pizzichini et al (15) for

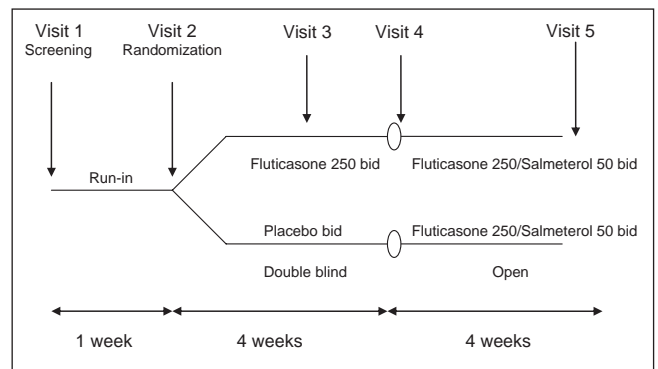


Figure 1) Study design and procedures performed at each visit. Visit 1: Spirometry pre- and postbronchodilator and sputum induction. Visit 2: Skin testing to common allergens, Asthma Control Questionnaire (ACQ) and Asthma Quality of Life Questionnaire (AQLQ), spirometry, methacholine challenge and sputum induction. Visit 3: Spirometry, ACQ and AQLQ. Visit 4: ACQ and AQLQ, spirometry, methacholine challenge and sputum induction. Visit 5: ACQ and AQLQ, spirometry and sputum induction. bid Twice daily

total and differential cell counts. All slides were read at a single site (Sacré-Coeur Hospital) by the same technologist.

5. The methacholine inhalation test was performed using the method described by Juniper et al (16), and the results were expressed as the PC_{20} in noncumulative units.

Statistical analysis

Data were reported as mean and SD, except for PC_{20} , which was reported as geometric mean and geometric SD. Sputum cell counts were reported as medians and interquartile ranges. Comparisons within groups were performed using a paired *t* test, whereas comparisons between groups were performed using a Student's *t* test except for the sputum results, which were analyzed using a Wilcoxon test or a Mann-Whitney test.

The primary study outcome was the change in the ACQ score, which was deemed to be the most relevant clinical measure to assess asthma control over the study period. A change in ACQ of 0.5 has been shown to be clinically relevant (17). Secondary outcomes included results from the AQLQ, and changes in FEV_1 and PC_{20} . Statistical significance was set at $P < 0.05$. The analysis was performed using SPSS version 16.0 (IBM Corporation, USA).

RESULTS

Subject characteristics

Seventy subjects were screened for the present study, of whom 58 were excluded. The majority of excluded subjects had either a sputum eosinophil count $> 2\%$ ($n=18$) or an ACQ score < 2 at both baseline visits ($n=18$). The remaining 12 eligible subjects completed the study. No subject experienced an asthma exacerbation during the study.

Six subjects were randomly assigned to receive fluticasone propionate (Diskus® 250 µg) one inhalation twice daily, and six received placebo Diskus® one inhalation twice daily. This was followed by an open-label treatment phase comprising fluticasone propionate 250 µg/salmeterol 50 µg combination inhaler, one inhalation twice daily for four weeks.

The demographic characteristics of both groups are presented in Table 1. Eighty-three per cent (five of six) of patients in both randomized groups were atopic. Overall, there was no significant reversibility in FEV_1 after bronchodilator ($6.9 \pm 10.8\%$) in the ICS group, nor in the control group ($3.5 \pm 13.9\%$). The methacholine challenge was not performed in two subjects at visit 2 due to moderate or severe airflow limitation. Both subjects showed postbronchodilator FEV_1 reversibility

TABLE 1
Baseline characteristics of subjects randomly assigned to inhaled corticosteroid (ICS) or placebo

	Treatment	
	ICS (n=6)	Placebo (n=6)
Sex, male/female, n/n	1/5	4/2
Age, years	38.0±15.3	43.0±11.7
Duration of asthma, years	19.3±21.4	24.2±17.4
Body mass index, kg/m ²	28.1±9.0	25.4±6.8
Atopic/nonatopic, n/n	5/1	5/1
Smoking habit, exS/NS, n/n	2/4	2/4
Pack-years	1.7±3.2	1.0±2.3

Data presented as mean ± SD unless indicated otherwise. exS Ex-smoker; NS Nonsmoker

TABLE 2
Changes in symptoms, lung function and airway inflammation after four weeks of inhaled corticosteroid (ICS) treatment or placebo

	ICS group (n=6)			Placebo group (n=6)			P†
	Baseline	Week 4	P*	Baseline	Week 4	P*	
SABA [‡] , puff/day	1.5±1.5	0.6±1.1	0.2	3.2±2.6	3.3±2.6	0.8	0.4
ACQ [‡]	2.4±0.7	1.4±0.5	0.007	2.3±0.3	2.2±0.7	0.6	0.008
AQLQ [‡]	4.3±1.1	5.5±1.2	0.01	4.5±1.2	4.8±1.1	0.3	0.056
FEV ₁ [‡] , L	2.3±0.5	2.4±0.4	0.4	2.9±0.4	2.7±0.4	0.04	0.02
FEV ₁ [‡] , % predicted	77.0±8.2	78.9±10.7	0.4	79.7±12.1	74.4±14.2	0.04	0.02
PC ₂₀ [‡] , mg/mL	0.7±5.6	2.4±3.4	0.01	0.9±3.4	1.9±8.9	0.2	0.7
TCC [§] , ×10 ⁶ /g	3.4 (5.7)	2.5 (2.6)	0.3	3.1 (2.6)	5.3 (11.0)	0.3	0.2
Eos [§] , %	0.8 (0.4)	0.3 (0.6)	0.2	0.5 (1.3)	0.3 (1.0)	0.7	0.8
Neu [§] , %	78.4 (64.2)	27.9 (3.4)	0.03	64.8 (44.1)	70.3 (50.3)	0.7	0.2

*Values refer to the within-group comparison between baseline (visit 2) and week 4 (visit 4); †Values refer to the between-group comparison of the changes between visit 2 and visit 4; ‡Data presented as mean ± SD; §Data presented as median (interquartile range). ACQ Asthma Control Questionnaire; AQLQ Asthma Quality of Life Questionnaire; Eos Eosinophils; FEV₁ Forced expiratory volume in 1 s; inh Inhalation; Neu Neutrophils; PC₂₀ Provocative concentration of methacholine causing a 20% fall in FEV₁; SABA Short-acting beta₂ agonist; TCC Total cell count

>12%. Two subjects in each group had a sputum eosinophil count of between 1% and 2%; free eosinophil granules were present in the sputum of only one subject in the treated group.

Changes in asthma symptoms and lung function within and between groups after a four-week treatment with inhaled fluticasone or placebo

After four weeks of treatment with inhaled fluticasone, there was a statistically significant and clinically important improvement in the primary outcome – namely, the ACQ score. In the ICS-treated group, a decrease of 1.0±0.5 in the ACQ score was observed, whereas no significant change in the placebo-treated group occurred (decrease of 0.09±0.4) (P=0.008) (Table 2, Figure 2). An improvement in the ACQ score was observed at two weeks after the start of the ICS treatment (1.7±0.8) compared with baseline (2.4±0.7) (P=0.007), but only in the ICS-treated group. A clinically relevant improvement in the AQLQ scores (1.2±0.8) was observed in the ICS-treated group compared with the placebo-treated group (0.3±0.7) (P=0.056). An improvement in airway responsiveness after treatment in the ICS group was observed, but this improvement (increase of 3.4±0.6 doubling doses of methacholine) was not significantly different from that observed in the placebo group (increase of 2.1±2.6 doubling doses of methacholine) (P=0.7). There was a greater decrease in the FEV₁ (−0.2±0.2 L) in the

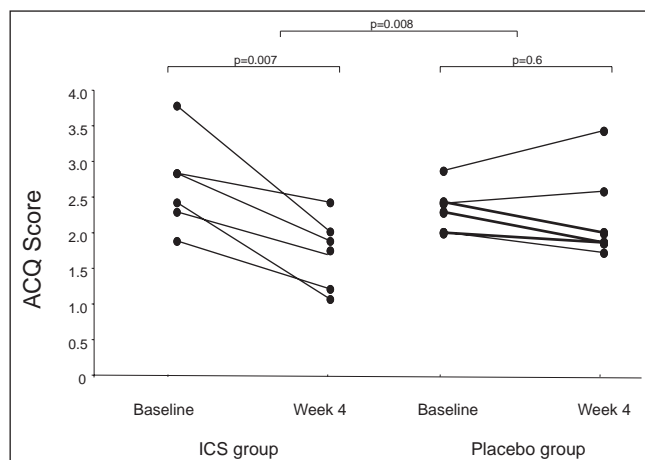


Figure 2) Changes in the Asthma Control Questionnaire (ACQ) score after treatment with inhaled corticosteroids (ICS) or placebo

TABLE 3
Changes in symptoms, lung function and airway inflammation after four weeks of treatment with inhaled corticosteroid (ICS) (combination of fluticasone and salmeterol)

	ICS group (n=6)			Placebo group (n=6)		
	Week 4	Week 8	P*	Week 4	Week 8	P*
SABA [‡] , inh/day	0.6±1.1	0.5±0.8	0.5	3.3±2.6	1.6±2.9	0.1
ACQ [‡]	1.4±0.5	1.3±1.1	0.7	2.2±0.7	1.6±1	0.03
AQLQ [‡]	5.5±1.2	5.7±1.5	0.5	4.8±1.1	5.2±1	0.1
FEV ₁ [‡] , L	2.4±0.4	2.5±0.6	0.07	2.7±0.4	3.1±0.5	0.05
FEV ₁ [‡] , % pred	78.9±10.7	84.3±14.0	0.2	74.4±14.2	85.1±12.3	0.05
TCC [‡] , ×10 ⁶ /g	2.4±3.4	1.4 (8.9)	0.4	5.3 (11.0)	3.8 (13.4)	0.7
Eos [‡] , %	0.3 (0.6)	0.1 (0.9)	1	0.3 (1.0)	0.3 (0.5)	0.3
Neu [‡] , %	27.9 (3.4)	32.4 (24.4)	0.7	70.3 (50.3)	77.0 (74.7)	0.6

*Values refer to the within-group comparison between week 4 (visit 4) and week 8; †Data presented as mean ± SD; ‡Data presented as median (interquartile range). ACQ Asthma Control Questionnaire; AQLQ Asthma Quality of Life Questionnaire; Eos Eosinophils; FEV₁ Forced expiratory volume in 1 s; inh Inhalations; Neu Neutrophils; pred Predicted; SABA Short-acting beta-2 agonist; TCC Total cell count

placebo-treated group compared with the ICS-treated group (0.04±0.1 L) following four weeks of treatment (P=0.02) (Table 2). A significant decrease in the number of sputum neutrophils was only observed in the ICS-treated group (Table 2).

Changes after treatment with ICS and LABA combination therapy
After one month of open-label treatment with the fluticasone/salmeterol combination inhaler, only subjects who were initially treated with placebo during the first phase of the study showed a significant improvement in ACQ score and FEV₁. No additional improvement was observed in the group who were previously treated with ICS (Table 3).

DISCUSSION

The present study showed significant improvements in asthma control and disease-specific quality of life in noneosinophilic asthmatic subjects treated with 500 µg of inhaled fluticasone daily for four weeks compared with noneosinophilic asthmatic subjects treated with placebo for the same time period. This effect was observed in a population of nonsmoking asthmatic subjects mainly composed of atopic subjects (83.3%).

These results were consistent with our previous findings from an uncontrolled study (6), as well as with the results of a recent study showing a significant improvement in asthma symptoms and bronchial

hyper-responsiveness in more than 40% of the noneosinophilic asthmatic subjects treated with ICS (18). However, these results differ from other studies that showed a poor response to ICS in patients with noneosinophilic asthma (4). To our knowledge, only one other randomized controlled trial studying the response to ICS in noneosinophilic asthmatic subjects has been performed to date (5). That trial was a crossover study comparing eight weeks of 400 µg daily of inhaled mometasone versus placebo in 11 steroid-naïve subjects. The authors did not find any statistically significant improvement in symptom score, FEV₁, AQLQ score or PC₂₀ after ICS or placebo treatment in noneosinophilic asthmatic subjects. However, one of the inclusion criteria of that study was an ACQ >1.57, but the changes in the ACQ score were not reported and were not one of the outcomes of interest. This group of subjects also underwent bronchial biopsies and showed a low number of eosinophils in the airway mucosa. Because we previously showed that subjects with low sputum eosinophil counts had a significant number of eosinophils in the airway mucosa (19), we cannot be sure whether our subjects did not have a high number of eosinophils in the airway mucosa explaining a beneficial effect of the treatment with ICS. One of the main differences between both study groups was the large proportion of atopic subjects in our group (83%) compared with the other study (18%). The response to ICS may be different in atopic noneosinophilic subjects compared with nonatopic subjects.

Several hypotheses may be offered to explain the corticosteroid responsiveness of our subjects. Noneosinophilic asthma may represent a heterogeneous group in which there are steroid responders or non-responders, as suggested by a previous study (18).

In healthy adult subjects, the mean, median and 90th percentile of sputum eosinophils have been shown to be 0.4%, 0.0% and 1.1%, respectively (20). Therefore, a sputum eosinophil count of between 1% and 2% may still represent some degree of airway inflammation responsive to ICS. Finally, a recent study showed that the eosinophil protein content of airway macrophages seems to be a reliable marker of persistent eosinophilic inflammation, independent of sputum eosinophil count (21). It is possible that in spite of a low sputum eosinophil percentage, our subjects may have had a substantial proportion of macrophages containing eosinophil proteins.

We found a high sputum neutrophil count in both groups at baseline before treatment. This is consistent with previous observations that subjects with noneosinophilic inflammation tend to have a neutrophilic inflammation (22). Surprisingly, we observed that a significant decrease in the number of neutrophils only occurred in the ICS-treated group. The placebo-treated group did not show any significant decrease in neutrophil counts. The relevance of this finding remains unclear.

We did not observe any further change in asthma control or lung function in the ICS-treated group after the introduction of a LABA.

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Our results are consistent with a meta-analysis (23) showing that the combination of ICS/LABA does not bring any additional benefit compared with ICS alone in steroid-naïve subjects. However, the group who received the placebo in the initial phase of the study had a significant improvement in their ACQ score and their FEV₁ after one month of treatment with combination ICS and LABA, confirming the beneficial effect of treatment with ICS in noneosinophilic asthmatic patients. It should be noted that definitive conclusions cannot be derived from our study concerning the effects of ICS/LABA, given that the treatment was administered in an open-label extension to our primary study and because our study was relatively underpowered to assess the additional effects of LABAs when added to ICS in this population.

We encountered a major challenge in the recruitment of the study subjects. Our initial sample size calculation had envisioned enrolling 50 subjects in each group to detect a change of 0.5 in the ACQ score between groups. However, despite major recruitment efforts, we managed to recruit only 12% of our original sample size. Many mildly uncontrolled asthmatic patients had sputum eosinophilia. The population of uncontrolled noneosinophilic steroid-naïve asthmatic subjects is likely to be small.

CONCLUSION

We have demonstrated a significant improvement in asthma quality of life and asthma control after treatment with ICS in subjects with low eosinophil count. Although the response to ICS is greater in asthmatic subjects with an eosinophilic inflammatory phenotype compared with a noneosinophilic inflammatory phenotype (5,18), a low sputum eosinophil count should not prevent clinicians from initiating treatment with an ICS in uncontrolled asthmatic subjects.

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