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ORIGINAL ARTICLE

Therapeutic potential of hydroxychloroquine on serum B-cell activating factor belonging to the tumor necrosis factor family (BAFF) in rheumatoid arthritis patients

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Abstract *Objective:* To assess the serum B-cell activating factor belonging to the tumor necrosis factor family (BAFF) level in rheumatoid arthritis (RA) patients in view of different treatment regimens received and evaluate its relation with disease activity.
Patients and methods: Ninety female RA patients were included. Sixty were on disease modifying anti-rheumatic drugs (DMARDs); 34 on hydroxychloroquine (HCQ) plus methotrexate (MTX), 26 on leflunomide (LFN) plus MTX and 30 newly diagnosed cases not yet on any treatment. Thirty age and gender matched healthy subjects served as controls. Full history taking, clinical examination and relevant laboratory investigations were performed. Disease activity score, in 28 joints (DAS-28), was calculated.
Results: Serum BAFF level was significantly higher in patients (1.82 ± 0.91 ng/ml) compared to control (0.71 ± 0.33 ng/ml; $p < 0.001$). There was a significantly lower BAFF and disease activity in patients receiving DMARDs (1.55 ± 0.73 ng/ml and 3.08 ± 0.73) compared to new cases (2.36 ± 1.02 ng/ml and 3.46 ± 0.82) ($p < 0.001$ and $p = 0.036$, respectively). Those receiving HCQ + MTX had a lower BAFF level (1.29 ± 0.51 ng/ml) compared to those receiving

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LFN + MTX (1.94 ± 0.85 ng/ml; $p = 0.002$). The BAFF level significantly correlated with the presence of anti-CCP antibodies, DAS28 and MTX dose in all RA patients ($r = 0.24$, $p = 0.02$; $r = 0.504$, $p < 0.001$; $r = 0.51$, $p < 0.001$, respectively). Only DAS28 and MTX dose would highly influence the BAFF level ($p = 0.015$ and $p = 0.001$, respectively).

Conclusion: Elevated level of BAFF in RA has been confirmed with a notable relation to disease activity making it a promising marker. The beneficial effect of hydroxychloroquine in dampening BAFF level throws light on the importance of considering it in combination among the newly developed biologics that also target B-cells.

1. Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory rheumatic disease, in which autoantibodies are part of the early disease manifestations. A pathogenic involvement of B cells is implicated in RA.[1] It is a systemic autoimmune arthritis that clinically manifests as joint pain, stiffness and swelling. If left untreated, persistent synovitis can progress to cartilage and bone destruction; ultimately to major long-term disability and mortality. Disease-modifying antirheumatic drugs (DMARDs), such as methotrexate (MTX), leflunomide (LFN) and hydroxychloroquine (HCQ), have markedly improved clinical symptoms and slowed joint damage. Despite their effectiveness, some patients continue to have clinical manifestations and progressive joint destruction. Advances in the understanding of the pathogenesis of RA have led to the identification of novel cellular and molecular therapeutic targets. Biologic agents aimed at these targets have provided some evidence of effectiveness that is transforming the management of RA.[2] Current strategic regimens which concentrate on systematic ways to bring patients into remission all include MTX as the first choice.[3]

Although the specific trigger of the autoimmune response in RA is not known, pathogenesis is generally believed to be associated with the generation of autoantibodies through interactions of antigen-presenting cells with the adaptive immune system (T and B cells). The main inflammatory mediators of joint inflammation and destruction in RA are tumor necrosis factor alpha (TNF-α), interleukin-1 (IL-1), IL-6, chemokines, and proteases.[2] Interest in B-cells has been revived due to the description of new functions. Supporting a role for B-cells in the genesis of autoimmune diseases is the fact that the B-cell activating factor of the TNF ligand family (BAFF) is essential in their physiology. Based on experiments in mice, and validated in humans, this new cytokine has been highlighted. Excessive production of BAFF alters immune tolerance by rescuing self-binding B-cells. Overexpression in mice leads to autoimmune manifestation, and BAFF levels are elevated in the serum of autoimmune patients.[4] Since BAFF has been identified as a critical and major regulatory factor for B cell maturation and survival, convincing evidence indicates that deregulation of BAFF is involved in the pathogenesis of B-cell related autoimmune diseases including RA,[5–7] Juvenile idiopathic arthritis (JIA),[8] SLE,[9,10] Systemic sclerosis (SSc)[10] and Behcets disease.[11] Blockade of BAFF activity significantly improves the symptoms of autoimmune diseases such as systemic lupus erythematosus (SLE) and RA both in animal models and clinical trials.[5] Advances in our understanding of the BAFF system offer the opportunity to improve our therapeutic approach.[4]

During the past century, many immunosuppressive drugs have been described. Often their mechanisms of action were established long after their discovery.[12] Advances in our understanding of the key cells and inflammatory cytokines have led to the development of targeted biologic agents.[2] Biologic therapies have profoundly changed the course of RA, but factors that predict response, which could be used to optimize the use and selection of these costly agents that have potentially severe side effects, have not been identified. Because B-cells play critical roles in RA, developing serum biomarkers of B cell activation are potential predictive factors for the efficacy of biologic agents targeting B cells.[13]

The management of RA has entered a new era with the arrival of biologic agents. However, the tools needed to predict response to these drugs to allow tailoring of the treatment regimens are still lacking. This apparent gap in knowledge may lead to the serial use of several immunosuppressive drugs, which may expose patients to an elevated risk of adverse side effects.[13]

Biologic agents are effective in reducing clinical signs of inflammation in RA patients who have failed DMARDs and significant benefits of their combination with MTX have been documented. All biologic agents carry an increased risk of infections with an additional potential side effect of site reactions. Patients being considered for biologic agents should be screened annually for tuberculosis and should receive pneumococcal, influenza, and hepatitis B vaccinations.[2]

Methotrexate (MTX) is currently the most frequently used drug in the treatment of RA. The drug had been synthesized in 1948 and first tests to treat patients with psoriasis and RA were published in 1951. However, until the 1980s there was only limited use of MTX in the treatment of RA. Since the 1990s MTX is the DMARDs of first choice for the treatment of RA in most countries worldwide. By definition, DMARDs in RA are those compounds for which an inhibiting effect on radiographic progression has been demonstrated. Several combinations of DMARDs have been tested, most commonly with MTX as the anchor drug. There are now three main combinations that are playing an important role: MTX + HCQ, MTX + LFN and MTX + biologics such as anti-TNF and other new compounds which block IL6 receptor or T-cell activation and delete B cells.[3] Emerging data from further studies provide critical insight regarding the role of B cells and autoantibodies in various autoimmune conditions and will guide the development of more efficacious therapeutics and better patient selection.[14]

The aim of the present study is to assess the serum BAFF level in RA patients in view of different treatment regimens received and evaluate its relation with the disease activity.

2. Patients and methods

2.1. Study design

Ninety female patients with definite RA diagnosed according to the 2010 ACR/EULAR RA classification criteria[15] were recruited from the Rheumatology and Internal medicine outpatient clinics of Cairo University Hospitals and included in this study. Patients on a stable dose of combination therapy for the preceding 6 months were grouped into 34 on HCQ (400 mg/day) + weekly intramuscular (IM) MTX and 26 on LFN (20 mg/day) + weekly IM MTX. Thirty newly diagnosed female RA cases not yet on any basic therapy were included and 30 age and gender matched healthy subjects served as controls. Full history taking and thorough clinical examination were performed for all patients. Laboratory investigations in the form of complete blood count (CBC), erythrocyte sedimentation rate (ESR), rheumatoid factor (RF) and anti-cyclic citrullinated peptide (anti-CCP) were performed. Disease activity score, in 28 joints (DAS-28),[16] was calculated. None of the patients received any biologic agent before and those receiving corticosteroids for the last 6 months or receiving any other combination therapy were also excluded. None of the patients had any associated other rheumatic disease or chronic inflammatory disease affecting other systems. Informed consents were taken from the patients and the study was approved by the local ethics committee and has been carried out in accordance with the declaration of Helsinki.

2.2. Determination of serum BAFF level

All sera were preabsorbed with protein A (Amersham Biosciences, Piscataway, NJ, USA) to deplete Ig. Serum BAFF levels were analyzed by Enzyme Linked Immunosorbent Assay (ELISA) using commercially available kits according to the manufacturer's protocol (Quantikine; R&D System, Minneapolis, MN, USA).

2.3. Statistical analysis

The data were collected, tabulated and analyzed by SPSS package version 15 (SPSS corporation, USA). Data were summarized as mean ± SD. Mann–Whitney test was used for comparative analysis of 2 quantitative data. Non-parametric analysis of variance (Kruskall–Wallis) was used for comparison of more than two groups; Spearman's correlation analysis was used for detection of the relation between 2 variables. Logistic regression analysis was applied to detect the predictors for the elevated BAFF level. Results were considered significant at $p < 0.05$.

3. Results

The mean age of the RA patients (36.08 ± 6.25 years) was comparable with that of the control (37.23 ± 6.95 years, $p = 0.72$).In the RA patients, the ESR was significantly higher (56.29 ± 18.7 mm/1st h) and the hemoglobin content was lower (11.25 ± 1.52 g/dl) compared to the control (25.27 ± 11.22 mm/1st h and 12.91 ± 1.26 g/dl) ($p < 0.001$ in both comparisons). The WBC and platelet counts were comparable between patients ($6.39 \pm 2.14 \times 10^3$/ml and $316.16 \pm 76.33 \times 10^3$/ml) and control ($6.78 \pm 2.55 \times 10^3$/ml and $290.37 \pm 68.57 \times 10^3$/ml, $p = 0.45$ and $p = 0.09$, respectively). The serum BAFF level was significantly higher in the RA patients (1.82 ± 0.91 ng/ml) compared to the control (0.71 ± 0.33 ng/ml) ($p < 0.001$).

There was a significantly lower BAFF and disease activity in RA patients receiving DMARDs (1.55 ± 0.73 ng/ml and 3.08 ± 0.73) compared to the newly diagnosed cases (2.36 ± 1.02 ng/ml and 3.46 ± 0.82) ($p < 0.001$ and $p = 0.036$, respectively). Also there was a significantly older age (37.33 ± 6.42 years) and longer disease duration (10.58 ± 3.64 years) in those on treatment compared to those not (33.6 ± 5.14 years and 1.78 ± 1.32 years; $p = 0.004$ and $p < 0.001$, respectively).

Comparison of the demographic and clinical features, disease activity, laboratory investigations and serum BAFF level of the RA patients on HCQ + MTX with those on LFN + MTX are shown in Table 1.

Patients on HCQ + MTX had a significantly higher BAFF (1.29 ± 0.51 ng/ml) and DAS28 (2.99 ± 0.71 years) than those not on any therapy (2.36 ± 1.02 ng/ml and 3.46 ± 0.82 years, $p < 0.001$ and $p = 0.017$, respectively). Those on LFN + MTX had a non-significant rise in the BAFF (1.94 ± 0.85 ng/ml) and DAS28 (3.21 ± 0.75) compared to those not receiving any therapy (2.36 ± 1.02 and 3.46 ± 0.82, $p = 0.1$ and $p = 0.25$, respectively). Serum BAFF levels in different study groups including RA patients on HCQ + MTX, on LFN + MTX, not receiving any therapy and the control, are shown in Fig. 1. Furthermore, the hemoglobin content significantly reduced in those not receiving any therapy (10.91 ± 1.46 g/dl vs 11.8 ± 1.42 g/dl, $p = 0.03$).

Correlation tests of serum BAFF with demographic features, MTX dose, disease activity and laboratory investigations are presented in Table 2.

Correlation of the serum BAFF level and the DAS28 in the RA patients is shown in Fig. 2. The BAFF significantly correlated with DAS28 in those on DMARDs: (HCQ + MTX: $r = 0.37$, $p = 0.026$ and LFN + MTX: $r = 0.45$, $p = 0.028$) and in those awaiting a therapeutic regimen ($r = 0.56$, $p = 0.001$).

A logistic regression analysis revealed that only DAS28 and MTX dose would highly influence the serum BAFF level ($p = 0.015$ and $p = 0.001$, respectively) among other independent factors including the age, disease duration and presence of anti-CCP.

4. Discussion

In the present study the serum BAFF level was significantly higher in the RA patients compared to the control. It was even higher in the newly diagnosed RA patients who had a higher disease activity and shorter disease duration. This was similar to the findings in other studies[1,6,17–20] that observed the increased levels and reported the important roles played by it in RA. The increased levels of BAFF in very early RA patients suggest that B-cell activation and the development of autoreactive B-cell responses might be crucial in early phases of the disease.[19–21]

Table 1 Comparison of the demographic and clinical features, disease activity, laboratory investigations and serum BAFF level of rheumatoid arthritis patients on HCQ + MTX with those on LFN + MTX.

Variable (mean ± SD)	RA patients on DMARDs (60)		*p* value
	HCQ + MTX (36)	LFN + MTX (24)	
Age (years)	38.33 ± 6.76	35.81 ± 5.68	0.13
Disease duration (years)	10.94 ± 3.93	10.04 ± 3.17	0.33
DAS28	2.99 ± 0.71	3.21 ± 0.75	0.28
Methotrexate (mg/week)	11.81 ± 4.21	15.94 ± 3.11	<0.001
ESR (mm/1st h)	53 + 19.57	54.63 ± 16.72	0.73
Hemoglobin (g/ml)	11.16 ± 1.58	11.8 ± 1.42	0.11
WBC ($\times 10^3$/ml)	6.28 ± 2.2	6.83 ± 2.37	0.37
Platelets ($\times 10^3$/ml)	302.06 ± 72.97	308.25 ± 68.07	0.74
RF positivity no. (%)	31(86.11)	21 (87.5)	0.88
Anti-CCP positivity no. (%)	26 (72.22)	19 (79.17)	0.54
Serum BAFF level (ng/ml)	1.29 ± 0.51	1.94 ± 0.85	0.002

DMARD: Disease modifying anti-rheumatic drug, DAS28: Disease activity score in 28 joints, ESR: Erythrocyte sedimentation rate, WBC: White blood cells, RF: Rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide, BAFF: B-cell activating factor belonging to the tumor necrosis factor family.

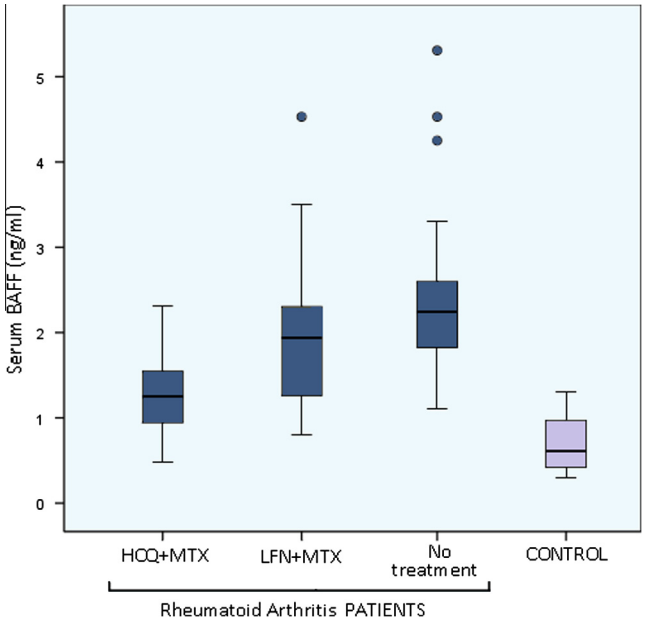

Figure 1 Serum BAFF level in different study groups including RA patients on HCQ + MTX, on LFN + MTX, not receiving any therapy and the control. BAFF: B-cell activating factor belonging to the tumor necrosis factor family, HCQ: hydroxychloroquine, MTX: methotrexate, LFN: leflunomide.

Table 2 Correlation of serum BAFF with demographic features, MTX dose, disease activity and laboratory investigations.

Variable	BAFF (ng/ml)	
	r	*p*
Age (years)	−0.2	0.06
Disease duration (years)	−0.18	0.09
DAS28	0.504	<0.001
Methotrexate (mg/week)	0.512	<0.001
ESR (mm/1st h)	0.03	0.8
Hemoglobin (g/ml)	0.11	0.31
WBC ($\times 10^3$/ml)	−0.02	0.87
Platelets ($\times 10^3$/ml)	0.11	0.29
RF positivity no. (%)	0.16	0.12
Anti-CCP positivity no. (%)	0.24	0.02

DAS28: Disease activity score in 28 joints, ESR: Erythrocyte sedimentation rate, WBC: White blood cells, RF: Rheumatoid factor, anti-CCP: anti-cyclic citrullinated peptide, BAFF: B-cell activating factor belonging to the tumor necrosis factor family.

The BAFF system plays a specific and key role in the development of autoimmunity. Focus on BAFF and autoimmunity, driven by pharmaceutical successes with the recent approval of a novel targeted therapy Belimumab, has relegated other potential roles of BAFF to the background. Far from being SLE-specific, the BAFF system has a much broader relevance in infection, cancer and allergy.[22]

B cells play critical roles in RA pathogenesis as they are the source of RFs and anti-CCP autoantibodies, which contribute to IC formation in the joints. These cells are also efficient antigen-presenting cells and contribute to T-cell activation through expression of co-stimulatory molecules. B cells simultaneously respond and produce chemokines and cytokines that promote leukocyte infiltration into the joints, formation of ectopic lymphoid structures, angiogenesis and synovial hyperplasia.[19]

In the present study, a significant correlation was present between the serum BAFF level and the DAS28 in the RA patients. This would support the suggestion of considering BAFF as a biomarker of disease activity in RA. In agreement to this finding, other studies found similar significant correlation of BAFF with DAS28 in RA patients.[1,23] On the other hand, the study of Gottenberg et al.[21] found markers of B-cell activation, except BAFF, were associated with disease activity, RF and anti-CCP secretion in early RA. In a previous study on juvenile idiopathic arthritis cases, BAFF correlated only with the juvenile arthritis disease activity score in 27 joints (JADAS-27) and was significantly higher in oligoarticular onset patients with uveitis.[8]

Despite long-term usage of anti-inflammatory drugs in RA patients, increased BAFF, might suggest a propensity to overproduce these inflammatory mediators but whether this re-

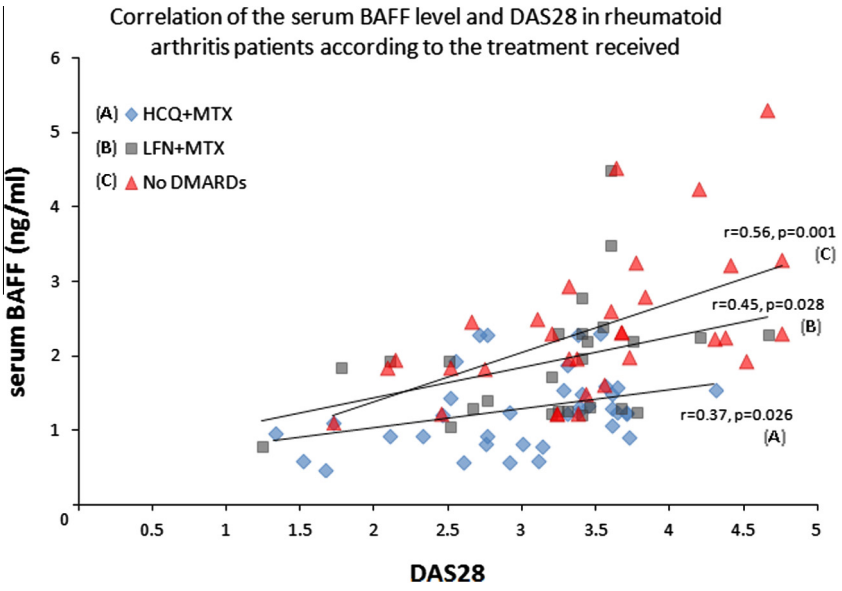

Figure 2 Correlation of the serum BAFF level and the DAS28 in all RA patients. BAFF: B-cell activating factor belonging to the tumor necrosis factor family, DAS28: Disease activity score in 28 joints.

sults from or contributes to greater disease activity remains moot.[24]

In this study, the BAFF level significantly correlated with the anti-CCP positivity in the RA patients but did not with the RF. In a different study, BAFF correlated with the titers of IgM RF and anti-cyclic citrullinated peptide autoantibody,[17] while in another recent study, the BAFF level had no correlation with the titers of RF and anti-CCP antibodies.

The BAFF level in RA patients receiving MTX combination therapy was significantly lower than in those still not receiving any treatment. In a follow-up study on methotrexate-treated early RA patients, reduced levels of BAFF, with parallel improvement in clinical activity and decrease in autoantibody titers were revealed.[17]

Patients of the present study on HCQ plus MTX combination therapy showed a significantly reduced serum BAFF level compared to those receiving LFN or those still not receiving any therapy. It has been reported in patients with primary Sjögren's syndrome (pSS) that the salivary and serum BAFF levels were significantly lowered when treated with HCQ.[25] Not only did HCQ alleviate symptoms and signs of dry eye but decreased tear fluid BAFF levels.[26] On the contrary, no significant change in serum BAFF level was observed in another study on pSS patients treated with low-dose corticosteroids or HCQ.[27] Hydroxychloroquine is still considered a standard drug, provided its use is optimized.[28]

There was a tendency to a reduced the BAFF level in RA patients on LFN plus MTX compared to those not receiving any medication. Leflunomide is an immunosuppressive drug that exerts its activity by inhibiting de novo pyrimidine synthesis by blocking the mitochondrial enzyme dihydro-orotate de hydrogenase (DHODH), and possibly, inhibition of tyrosine kinase activity.[29] It has been found to inhibit both T-cell and B-cell function[30] and a dose-dependent protective effect on autoantibodies production in tolerized mice was presented.[29] Consistent with the role of DHODH in lymphocyte proliferation, leflunomide has proven effective and was approved for the treatment of RA.[31] Significant benefits of short-term leflunomide therapy were associated with functional suppression of peripheral B lymphocytes.[32]

One of the main goals in management is to optimize the treatment for rheumatoid arthritis patients. Nevertheless, rigorous evaluation of the risk/benefit ratio of new drugs and of their most appropriate place in the therapeutic strategy is indispensable.[28]

Targeting BAFF might be a promising and useful therapeutic strategy to treat B-cell related autoimmune diseases[5] and in early RA.[19,21] A good clinical response to TNF antagonist therapy in RA patients is associated with a decline in BAFF levels. Increased BAFF expression in affected joints may contribute to ongoing disease activity, and reduction of such expression may help promote a favorable clinical response to the anti-TNF therapy.[18]

Rituximab results appear very interesting and other anti-BAFF drugs should also be evaluated.[28] Tabalumab, a human monoclonal antibody that neutralizes membrane-bound and soluble BAFF in RA patients with inadequate response to MTX, showed a dose-response improvement in disease activity assessed by DAS28[33] and a comparable safety profile to those receiving placebo with MTX.[34] Belimumab is another fully human monoclonal antibody that inhibits BAFF and it is being developed for the treatment of RA. It was well tolerated over 24 weeks and significantly increased the American College of Rheumatology (ACR)20 responses, especially in patients with high disease activity, positive RF, no anti-TNF treatment experience and those who had failed MTX therapy. However, belimumab failed to demonstrate significantly improved ACR50 and ACR70 responses in the single Phase II clinical trial of RA. Expert opinion suggested that its clinical efficacy needs to be further investigated in future clinical trials and that careful patient selection may be necessary to achieve optimal clinical outcomes in RA.[6]

The BAFF assay was a foremost predictor of "good-EULAR response" in RA and should be identified as a possible biomarker of response in patients undergoing the B cell depletion therapy (BCDT).[35]

It is important that hydroxychloroquine is not missed from the new therapeutic armamentarium targeting B-cells and further studies combining HCQ to anti-B cells might improve the outcome of the disease. Further longitudinal studies on a larger number of patients and including those on different new biologic agents would be interesting to confirm the present results and to throw light on the importance of considering the classic treatment regimens for RA in combination with the biologics when first line medications fail to achieve a satisfactory response. The BAFF should be considered as a therapeutic target among the many options of RA treatment.

5. Conclusions

In conclusion, the elevated level of BAFF in RA has been confirmed with a notable relation to disease activity making it a promising marker. The beneficial effect of HCQ in dampening the serum BAFF level throws light on the importance of considering it in combination among the newly developed biologics that also target B-cells.

6. Conflict of interest

The authors declare none.

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