

## Utility of Monitoring Azathioprine Metabolites in the Management of Children with Autoimmune Hepatitis

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### Abstract

**Aim:** Although monitoring of the active metabolite (6-thioguanine or 6-TG) and hepatotoxic metabolite (6-methylmercaptopurine or 6-MMP) of the drugs azathioprine (AZA) or 6-mercaptopurine is well-established in children with inflammatory bowel disease, there is little information about the utility of this practice in children with AIH.

**Objectives:** The purpose of this single center retrospective study was two-fold: 1) To determine if metabolite monitoring (MM) was associated with improved clinical outcome and 2) To determine levels of 6-TG associated with remission.

**Methods:** Chart review was performed of all patients ages 0-21 years at the Johns Hopkins Hospital with definite or probable AIH from 1991 to 2012 seen over two years of follow up.

**Results:** Twenty-one patients with AIH met the inclusion criteria of pre-transplant state and treatment with AZA or 6-MP. 10 patients did not have MM (Group 1); 11 patients had MM at least once (Group 2). Average AZA dose for Group 1 patients was 1.2 (0.6-1.8) mg/kg/day vs. 1.9 (1.3-2.9) for Group 2 patients ( $P=0.002$ ). 4/10 (40%) Group 1 patients achieved remission vs. 7/11 (64%) Group 2 patients ( $P=0.39$ ). The average 6-TG level for Group 2 remission patients was  $162.7 \text{ pmol}/8 \times 10^8 \text{ red blood cells (RBC)}$  (41.5-316;  $N=7$ ). One patient developed liver failure presumably secondary to AZA-cholestasis (6-MMP level of  $6792 \text{ pmol}/8 \times 10^8 \text{ RBC}$ ), since it resolved with discontinuation of AZA.

**Conclusions:** MM in children with AIH may prove useful for determining 6TG levels associated with remission, permit dose escalation as necessary, and assist in determination of AZA toxicity.

**Keywords:** 6 Thioguanine (6TG); 6 Methylmercaptopurine (6MMP); Pediatric liver disease; Drug Monitoring; Children; Autoimmune Hepatitis; Hepatotoxicity

**Abbreviations:** AASLD: American Association for the Study of Liver Diseases; AIH: Autoimmune Hepatitis; ALT: Alanine Aminotransferase; ANC: Absolute Neutrophil Count; IAIHG: International Autoimmune Hepatitis Group; MM: Metabolite Monitoring; 6-MMP: 6-Methylmercaptopurine; RBC: Red Blood Cells; 6-TG: 6-Thioguanine; TPMT: Thiopurine Methyltransferase

### Introduction

Optimal treatment of children with autoimmune hepatitis (AIH) is still being defined. Conventional treatment of AIH in children consists of prednisolone (or prednisone) 2 mg/kg/day (maximum 60 mg/day), which is gradually decreased over a period of 4 to 8 weeks [1], but formal guidelines for children are lacking. In addition, azathioprine (AZA) is usually administered as a steroid-sparing agent at a dose of 0.5 to 2 mg/kg/day. The optimal duration of immunosuppressive treatment for AIH is unknown and the majority of patients require

chronic immunosuppression. Although testing for serum levels of AZA metabolites (6-thioguanine (6-TG) (the therapeutic component) and 6-methylmercaptopurine (6-MMP) (the hepatotoxic component) became commercially available in 1998, optimum levels in children with AIH have still not been determined [2]. There are conflicting reports as to the utility of measuring metabolite levels in children with inflammatory bowel disease [3-5]. Dubinsky et al. [4] reported that children with inflammatory bowel disease who achieve a level considered as therapeutic ( $235\text{-}450 \text{ pmol}/8 \times 10^8 \text{ red blood cells (RBC)}$ ) have improved outcome and decreased drug toxicity compared to children who do not. According to that study, the 6-MMP level which was considered potentially hepatotoxic was  $>5700 \text{ pmol}/8 \times 10^8 \text{ RBC}$ .

There have been small and conflicting reports as to the optimum drug levels in children with AIH [2,6]. Dhaliwal reported that adults with AIH who maintained remission had significantly higher concentrations of 6-TG ( $237$  versus  $177 \text{ pmol}/8 \times 10^8 \text{ RBC}$ ) vs. those who did not [7]. The purpose of this report was to test the hypothesis that children with AIH in whom metabolite monitoring (MM) was performed will exhibit higher response rates and less drug toxicity of azathioprine (hepatitis and neutropenia) vs. children without MM.

## Methods

### Human subjects

40 patients ages 0-21 years and diagnosed with AIH were enrolled into the Pediatric Liver Center at Johns Hopkins from 1991 to 2012, and identified from the Pediatric Liver Center database. Inclusion criteria were a probable or definite AIH diagnosis according to the revised International Autoimmune Hepatitis Group (IAIHG) scoring system [8] pre-transplant state and AZA/6-MP therapy for at least 6 months. Exclusion criteria included no AZA or 6-MP therapy, overlap syndrome and/or diagnosis score <10 according to the revised IAIHG scoring system, hepatic encephalopathy at presentation, de novo AIH post liver transplant and missing ALT data. Data elements were tabulated according to 2 different groups: Group 1 (no MM) and Group 2 (MM at least once). Data were accessed retrospectively from the electronic medical charts of all patients meeting eligibility criteria. Demographics, clinical features, laboratory and histological data were collected for 6 different time points: at the time of presentation, 6 months, 1 year and 2 years after presentation, at the end of therapy and 1 year after cessation of therapy. The follow up duration under AZA or 6-MP therapy ranged from 6 months to 2 years. 6-MP doses were converted to AZA equivalents by using a conversion factor of 2.08 [9]. RBC 6-TG and 6-MMP were measured periodically during the two year follow up for Group 2 patients, with a mean of 2 times per patient (1-3). Average values for each patient were used in the analysis. RBC 6-TG and 6-MMP were measured by quantitative high pressure liquid chromatography with separate stationary and mobile phase for each nucleotide in peripheral RBC (Prometheus Laboratories, San Diego, CA).

Treatment regimens were based on the 2010 American Association for the Study of Liver Diseases (AASLD) Guidelines [1], with prednisone 2 mg/kg/day (maximum 60 mg/day) gradually tapered down as soon as the serum aminotransferases normalized. In the same time, AZA was administered at a dose of 0.5 to 2 mg/kg/day. Outcome was characterized in 4 ways according to AASLD guidelines: (1) remission (normal alanine aminotransferase (ALT) level), (2) incomplete response (abnormal ALT level but no worsening of clinical, laboratory and histological remission), (3) treatment failure (worsening of initial condition) and (4) drug toxicity. No treatment failure outcome was observed in this study, probably because of the exclusion of patients if hepatic encephalopathy was observed at the onset of the disease. Treatment withdrawal was attempted if no clinical or biochemical disease activity had been observed for a 2 year period.

The study protocol was approved by the Johns Hopkins Institutional Review Board which granted exemption for the need to obtain signed informed consent given that the study was a retrospective chart review with de-identified clinical and laboratory data.

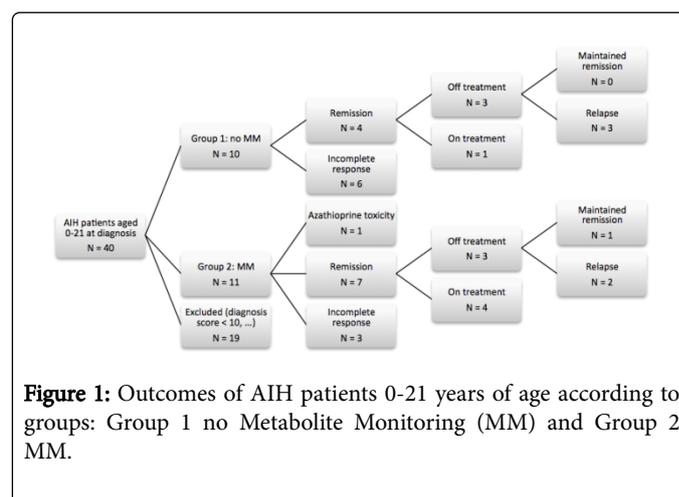
### Statistical analysis

Statistical analysis was performed using SPSS 21.0 (IBM SPSS Statistics for software Windows version 21.0 Armonk, NY: IBM Corp) and Graphpad Prism 5.0 (GraphPad Software, La Jolla, CA, USA). The averages of the AZA doses, 6-TG and 6-MMP concentrations for each patient over the study period were used in the analysis. All results are

presented as median (range). The Fisher's exact probability test was used to compare dichotomous variables, and the unpaired t-test was used to compare differences in the means of continuous variables. Correlations were assessed by Pearson's rank-correlation coefficient. A two-tailed p-value <0.05 was taken to indicate statistical significance in all analysis.

## Results

40 patients with AIH were identified of whom 21 met the inclusion criteria of pre-transplant state and treatment with AZA or 6-MP. The other 19 patients were excluded for different reasons: no AZA or 6-MP therapy (N=8), overlap syndrome and/or diagnosis score <10 according to the revised IAIHG scoring system (N=5), hepatic encephalopathy at presentation (N=4), de novo AIH after liver transplantation (N=1) and missing ALT data (N=1). Patient outcomes are represented in Figure 1. 4/10 (40%) Group 1 patients achieved remission under immunosuppressive therapy vs. 7/11 (64%) Group 2 patients (P=0.39).



**Figure 1:** Outcomes of AIH patients 0-21 years of age according to groups: Group 1 no Metabolite Monitoring (MM) and Group 2 MM.

### Comparison of groups with and without metabolite monitoring

Among the 21 included patients, 10 did not have MM (Group 1) and 11 had MM at least once (Group 2). Demographical, clinical and histological data for the 21 patients are summarized in Table 1. Average AZA dose were significantly higher in Group 2 patients (1.9 mg/kg/day, range 1.3-2.9) vs. 1.2 mg/kg/day, range 0.6-1.8 in Group 1, P=0.002). Otherwise there were no other significant differences between the two groups at baseline. Data points throughout the two years were available for the following numbers of patients in Groups 1 and 2 respectively: presentation (10 vs. 11); 6 months (9 vs. 9); 12 months (6 vs. 8) and 24 months (4 vs. 8) Laboratory values (ALT, AST, alkaline phosphatase, ANC) did not differ between groups at any of the time points assessed over the two year period. For example, values for ALT for the two groups were respectively 61 (22-154) vs. 45 (17-2360) U/L at 6 months for Groups 1 & 2; 59 (19-83) vs. 28 (13-1034) U/L at 12 months and 49 (17-291) vs. 26 (10-137) U/L at 24 months.

	Group 1 (N=10)	Group 2 (N=11)
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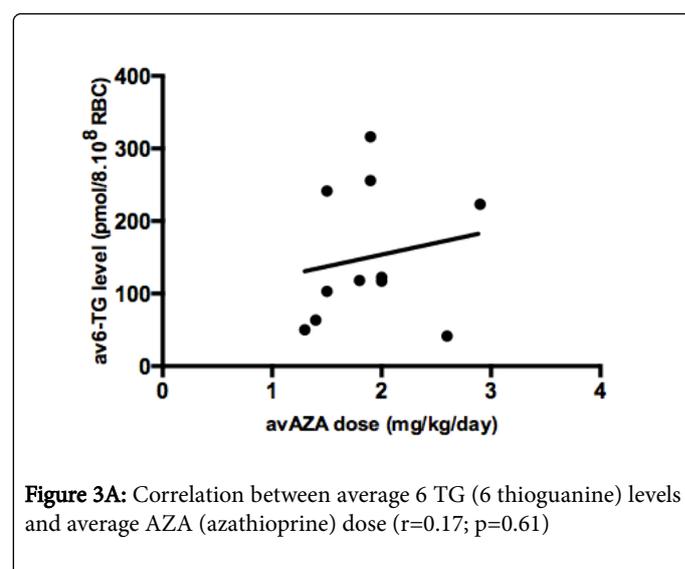
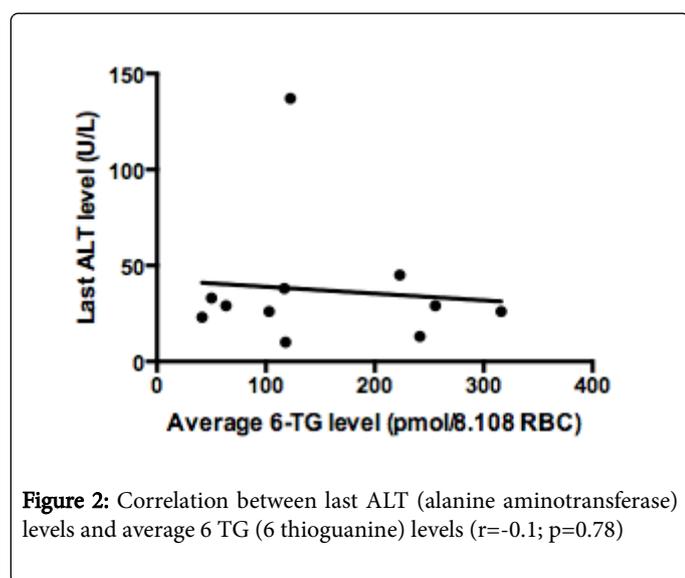
	No MM	MM
Male (#/%)	6 (60%)	7 (64%)
Age at diagnosis (years)	13.5 (3-18)	10 (1-15)
Duration of AIH at start of study (years)	1 (0-7)	1 (0-4)
Follow up (months)	23.5 (6-40)	25 (6-69)
Type I/II AIH	10 (100%)0	10 (91%)/1
Moderate-severe liver fibrosis at diagnosis	5 (50%)	5 (45%)
Average AZA dose (mg/kg/day)	1.2 (0.6-1.8)	1.9 (1.3-2.9)
Average prednisone dose (mg/kg/day)	0.4 (0.1-0.9)	0.4 (0.2-1.5)
Baseline ALT (U/L)	346 (62-1174)	120 (12-1110)
Baseline AST (U/L)	260 (32-1681)	90 (18-2458)
Baseline alkaline phosphatase (U/L)	246 (78-1020)	207 (83-595)
Baseline ANC (cells/ $\mu$ L)	3740 (1740-8730)	4760 (280-10250)

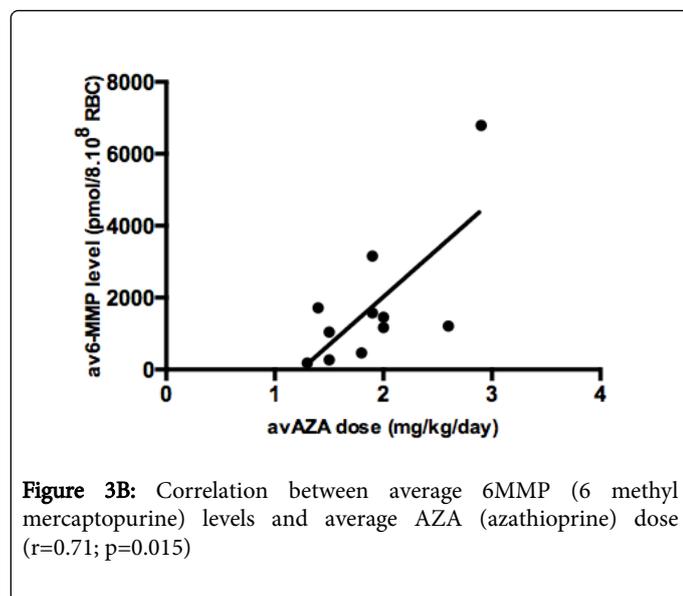
**Table 1:** Demographic and clinical characteristics of the 2 groups of AIH patients at baseline.

### Experience with metabolite monitoring

Thiopurine Methyl Transferase (TPMT) levels, average 6-TG levels and average 6-MMP levels in Group 2 patients were 29.7 U/mL RBC (normal TPMT level) (16.6-42.4), 118 pmol/ $8 \times 10^8$  RBC (41.5-316) and 1212 pmol/ $8 \times 10^8$  RBC (186-6792). The last ALT levels available for the patients did not correlate with average 6TG levels, ( $r=-0.1$ ;  $P=0.78$ ) (Figure 2). ALT levels used in Figure 2 are the last ALT levels collected during immunosuppressive therapy (after 2 years of therapy for 8/11 patients, after 1 year of therapy for 1/11 patient and after 6 months of therapy for 2/11 patients). One patient had an unusually high ALT level (137 U/L) after 2 years of therapy with an average 6-TG level of 122 pmol/ $8 \times 10^8$  RBC; his average AZA dose was 2 mg/kg/day and his TPMT level was intermediate.

The average 6-TG level for Group 2 remission patients was 162.7 pmol/ $8 \times 10^8$  RBC (41.5-316; N=7) versus 96.5 pmol/ $8 \times 10^8$  RBC (50-122.5; N=3) for Group 2 incomplete response patients ( $P=0.34$ ). The average AZA doses for Group 1 vs. Group 2 remission patients were 1.3 mg/kg/day (1.1-1.8) (N=4) vs. 1.8 mg/kg/day (1.4-2.6) (N=7),  $P=0.09$  and for the Group 1 vs. Group 2 incomplete response patients were 1.2 mg/kg/day (0.6-1.4) (N=6) vs. 2 mg/kg/day (1.3-2) (N=3),  $P=0.04$ . Comparing AZA doses for remission patients vs. incomplete response patients in Group 1, p-value was 0.24 and for Group 2 p-value was 0.91. There was no correlation between average 6-TG levels & average AZA doses ( $r=0.17$ ;  $P=0.61$ ); in contrast there was a strong positive correlation between the average 6-MMP levels & average AZA doses ( $r=0.71$ ;  $P=0.015$ ) (Figures 3A and 3B).





TMPT levels were measured for 2 patients in Group 1 and 7 patients in Group 2. Seven of those 9 patients had normal TPMT levels, 2 patients had intermediate TPMT levels (one from Group 1 & the other from Group 2), and no patient had low TPMT levels. Average AZA doses were not statistically different for normal TPMT levels patients (1.5 (1.1-2.9) mg/kg/day) comparing to intermediate TPMT levels (1.6 (1.1-2) mg/kg/day) (P=0.62) and missing TPMT levels (1.4 (0.6-2) mg/kg/day) (P=0.14).

Three patients had neutropenia (once). For 2 patients the TMPT levels are missing. One of those 2 patients had this neutropenia (1430 neutrophils/ $\mu$ l) when he was not under immunosuppressive therapy anymore. The other patient had an absolute neutrophil (ANC) count of 1360 neutrophils/ $\mu$ l once, while he was taking AZA 1.7 mg/kg/day (6-TG level=96 pmol/8  $\times$  10<sup>8</sup> RBC).

A third patient developed cholestasis treated with escalating doses of AZA (from 50 to 100 mg per day) by an outside physician who believed the patient was in progressive liver failure from AIH but did not monitor the AZA metabolite levels. Of note the patient had a normal TPMP level of 27 U/mL RBC. Her weight had increased, secondary to both increasing ascites and prednisone-induced obesity from 55.6 kg when the 6MP was initiated to 73.6 kg; thus the 6MP dose was 0.7 to 1.4 mg/kg/day or 0.9 to 1.8 mg/kg/day depending on which weight was used. This 16 year old female patient had evidence of end stage liver disease with ascites, coagulopathy (INR 1.4) and pancytopenia (white blood cells 2  $\times$  10<sup>9</sup>/L, ANC 0.28  $\times$  10<sup>9</sup>/L, hemoglobin 11.1g/dl, platelets 104  $\times$  10<sup>9</sup>/ $\mu$ l). Total bilirubin was 13.6 mg/dl and direct bilirubin 10.7. AST was 90 U/L and ALT was 120 U/L. Ammonia was 100 mcg/dl (normal 15-45). She was transferred to our hospital and listed for a liver transplant. Abdominal ultrasound revealed a nodular liver consistent with cirrhosis, splenomegaly and ascites. 6-TG level was 223 pmol/8  $\times$  10<sup>8</sup> RBC and she had normal TMPT activity; however 6-MMP level was 6792 pmol/8  $\times$  10<sup>8</sup> RBC, in the hepatotoxic range (>5700)(4). AZA was discontinued and the patient eventually recovered, was removed from the transplant list, and was eventually treated with low dose tacrolimus. Now 8 years later she is clinically well, has graduated from college, and is employed.

## Discussion

The main findings of our paper were as follows: 1) There was no statistically significant difference in outcome (remission rates) between the patients in whom drug metabolite levels were monitored and in those without monitoring 2) There was no statistically significant difference in 6-TG for remission/incomplete response patients 3) In patients in whom drug metabolites were monitored, AZA doses were higher vs. in those without monitoring and 4) AZA hepatotoxicity was closely correlated with AZA dose; 6-MMP drug levels were key to diagnosing apparent AZA hepatotoxicity in one patient in whom discontinuation of the drug reversed a potentially life-threatening condition and led to clinical resolution.

There are several interesting aspects of the remission achieved by our patients. Although remission rates were higher in those who were monitored (64%) vs. those who were not (40%), that difference was not statistically significant. Whether this lack of statistical significance meant that monitoring truly did not help achieve the goal of remission (similar to at least one report in patients with inflammatory bowel disease) [10] or whether the sample size was not large enough to detect a difference is not clear. We recognize that the small number of patients is a definite limitation of our study. However it is interesting that our patients with remission had lower 6-TG levels (163 pmol/8  $\times$  10<sup>8</sup> RBC) compared to levels thought to be therapeutic in children with inflammatory bowel disease (235-450 pmol/8  $\times$  10<sup>8</sup> RBC) [4] and lower than the levels thought to be therapeutic in adults with AIH (average 237 pmol/8  $\times$  10<sup>8</sup> pmol/RBC) [7].

It is of interest to compare our experience with that of Rumbo [2] et al. Her group demonstrated that, in general, drug metabolites could be utilized to help children with AIH safely achieve target levels consistent with those recommended for children with inflammatory bowel disease and were useful in assessing compliance to medication. Our approach was somewhat different in that our purpose was to achieve remission rather than to aim for a particular 6-TG level. With that approach we were able to demonstrate that remission can be achieved in children with AIH with average 6-TG levels lower than those targeted in children with inflammatory bowel disease. Somewhat similar to our observations, Nguyen et al. [6] reported that 32.6% of their group of children with AIH achieved remission with metabolite levels below those recommended for children with inflammatory bowel disease although 41.6% of the group who achieved remission actually had 6-TG levels above the therapeutic range for children with inflammatory bowel disease. Interestingly this group showed that there was no difference in 6-TG levels between children with active disease and those in remission, somewhat similar to our data showing no difference in 6-TG levels between children with remission and incomplete response. However we completely agree with both Rumbo et al. [2] and Nguyen et al. [6] that large multicenter studies will be necessary in order to optimize drug dosing in children with AIH.

Determination of factors determining remission rates in children with AIH is multi-factorial and, in addition to AZA dosing, includes factors such as compliance and possible overlap syndrome [11]. Ebbeson and Schreiber pointed out that, while application of the IAIHG scoring system in children with AIH is generally helpful, use of gamma glutamyl transpeptidase in place of alkaline phosphatase helps distinguish between those with overlap syndrome (who are less likely to achieve remission) and those with AIH [12]. We did not have gamma glutamyl transpeptidase available on all patients so chose to use alkaline phosphatase as per the IAIGHG, so in theory could have missed children with overlap syndrome.

Our data reinforce the observations of both Rumbo [2] and Nguyen [6] that monitoring of AZA metabolites may be most useful in both diagnosing and avoiding drug-related hepatotoxicity and neutropenia. We clearly demonstrate that there is a dose response between 6MMP and AZA whereas that was not the case with AZA dose and 6-TG. According to the data of Rumbo et al. [2], azathioprine related neutropenia corresponds to an ANC < 1000/ $\mu$ l & 6-TG > 450 pmol/ $8 \times 10^8$  RBC. None of our series of children ever had a 6-TG level this high. Our dramatic case of apparent AZA-related hepatotoxicity was in accordance with the observations of Dubinsky et al. [4] that a 6-MMP level > 5700 pmol/ $8 \times 10^8$  RBC) can be hepatotoxic.

In summary, our data suggest that at least a portion of children with AIH who are being treated with AZA can achieve remission with levels of 6-TG lower than those traditionally recommended for children with inflammatory bowel disease and that monitoring of AZA metabolites can assist in diagnosing and avoiding drug toxicity. In children with AIH who do not respond to conventional dose of AZA, serial monitoring of drug metabolites during cautious dose escalation may prove useful to achieve a therapeutic response and avoid drug toxicity, similar to the experience reported by Cuffari et al. [13] in patients with inflammatory bowel disease. Large scale prospective multi-center trials are clearly needed to optimize drug dosing in children with AIH.

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