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ORIGINAL ARTICLE

Male Endocrinology

Predictive factors for pituitary response to pulsatile GnRH therapy in patients with congenital hypogonadotropic hypogonadism

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Pulsatile gonadotropin-releasing hormone (GnRH) may induce spermatogenesis in most patients with congenital hypogonadotropic hypogonadism (CHH) by stimulating gonadotropin production, while the predictors for a pituitary response to pulsatile GnRH therapy were rarely investigated. Therefore, the aim of our study is to investigate predictors of the pituitary response to pulsatile GnRH therapy. This retrospective cohort study included 82 CHH patients who received subcutaneous pulsatile GnRH therapy for at least 1 month. Patients were categorized into poor or normal luteinizing hormone (LH) response subgroups according to their LH level (LH <2 IU l^{-1} or LH ≥2 IU l^{-1}) 1 month into pulsatile GnRH therapy. Gonadotropin and testosterone levels, testicular size, and sperm count were compared between the two subgroups before and after GnRH therapy. Among all patients, LH increased from 0.4 ± 0.5 IU l^{-1} to 7.5 ± 4.4 IU l^{-1} and follicle-stimulating hormone (FSH) increased from 1.1 ± 0.9 IU l^{-1} to 8.8 ± 5.3 IU l^{-1}. A Cox regression analysis showed that basal testosterone level (β = 0.252, *P* = 0.029) and triptorelin-stimulated FSH_{60min} (β = 0.518, *P* = 0.01) were two favorable predictors for pituitary response to GnRH therapy. Nine patients (9/82, 11.0%) with low LH response to GnRH therapy were classified into the poor LH response subgroup. After pulsatile GnRH therapy, total serum testosterone level was 39 ± 28 ng dl^{-1} versus 248 ± 158 ng dl^{-1} (*P* = 0.001), and testicular size was 4.0 ± 3.1 ml versus 7.9 ± 4.5 ml (*P* = 0.005) in the poor and normal LH response subgroups, respectively. It is concluded that higher levels of triptorelin-stimulated FSH_{60min} and basal total serum testosterone are favorable predictors of pituitary LH response to GnRH therapy.

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INTRODUCTION

Congenital hypogonadotropic hypogonadism (CHH) is a rare disease caused by gonadotropin-releasing hormone (GnRH) deficiency or dysfunction. It presents primarily with absent/partial pubertal development and infertility. Clinically, CHH patients are categorized into the anosmic type (Kallmann syndrome) or normosmic CHH type (nCHH) according to their olfactory status.[1] If spermatogenesis is required, pulsatile GnRH infusion effectively induces sperm production by promoting gonadotropin secretion from the pituitary gland.[2,3] Therefore, sufficient gonadotropin production in response to pulsatile GnRH therapy is a prerequisite for sperm induction.

About 10% of patients have a poor pituitary response to GnRH therapy,[4] indicating pituitary defects in the pathogenesis of CHH. However, it is not clear what factors may predict the pituitary response to GnRH therapy. Therefore, the purpose of our study was to investigate the possible pituitary defects in CHH patients and identify possible predictors of pituitary response by evaluating 82 CHH patients who underwent pulsatile GnRH therapy for at least 1 month.

PATIENTS AND METHODS

Patients

A diagnosis of CHH was made if a patient met all of the following criteria:[5] no pubertal development occurred before 18 years old; serum total testosterone was below 100 ng dl^{-1} (3.5 nmol l^{-1}) with low or inappropriate normal gonadotropin levels; other pituitary hormones were normal; the sellar magnetic resonance imaging (MRI) was negative; and other pathological conditions for secondary hypogonadotropic hypogonadism were excluded.

Patients were informed of all therapeutic choices: testosterone replacement therapy, pulsatile GnRH treatment, and combined gonadotropin therapy. Patients were free to switch from one treatment to another. Patients were followed up in Peking Union Medical College Hospital between January 2012 and December 2015.

Patients who met the following conditions were included in our study: (1) diagnosis of CHH was made; (2) no history of gonadotropin therapy, which typically contains human chorionic gonadotropin (HCG) and human menopausal gonadotropin (HMG); (3) no history of testosterone therapy or discontinued testosterone therapy for at least

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3 months; and (4) underwent pulsatile GnRH therapy for at least 1 month.

Patients were categorized into poor luteinizing hormone (LH) response subgroup (n = 9, LH <2 IU l^{-1}) or normal LH response subgroup (n = 73, LH ≥2 IU l^{-1}) according to their LH levels at 1 month of GnRH therapy. Levels of follicle-stimulating hormone (FSH), LH and testosterone, testicular size, and spermatogenesis rate were compared between the two subgroups.

Several cross-sectional studies have shown that the lower limit of normal LH range in male adults was about 2 IU l^{-1} by chemiluminescent methods.[6,7] Our own recent cross-sectional epidemiologic study, including 1034 healthy men, showed that the lower 2.5 and 5 percentiles of LH range were 1.65 IU l^{-1} and 1.93 IU l^{-1}, respectively. Therefore, in the present study, LH of 2 IU l^{-1} was chosen as the cutoff value for a normal pituitary response to GnRH therapy.

Clinical presentation, cryptorchidism history, and medical and family histories were recorded on each patient's first visit to hospital. Serum levels of gonadotropin and testosterone were measured and an MRI of the pituitary gland and olfactory bulb/tract was performed for each patient. Patients exhibiting dysplasia of the olfactory bulb/tract were diagnosed with Kallmann syndrome.

The study protocol was reviewed and approved by the ethics committee of the Peking Union Medical College Hospital. Written informed consent was obtained from each patient. The study was conducted in accordance with the Declaration of Helsinki.

Intervention and follow-up

Pulsatile gonadorelin (Ma'anshan Fengyuan Pharmaceutical Co, Anhui, China) was administered subcutaneously at a rate of 10 μg per 90 min (approximately 133 ng kg^{-1}), via a portable mini-pump (Shanghai Micro Invasive Life Technology Ltd. Co., Shanghai, China). Regular follow-up was conducted at an interval of 1–2 months throughout the therapy. GnRH dose was adjusted to maintain LH and FSH at 5–10 IU l^{-1} according to the following rules: if measured LH was lower than 2 IU l^{-1}, the dosage was increased 2 μg per pulse, to an upper limit of 16 μg GnRH per pulse. If measured LH was above 10 IU l^{-1}, the dosage was reduced 1 μg per pulse. Testicular size (measured by Prader orchidometer), FSH, LH and testosterone, and sperm count were measured at each visit. Semen samples were collected by masturbation and analyzed according to the standard World Health Organization method.[8] Successful spermatogenesis was defined as the presence of at least one sperm under microscopic observation after the seminal sample was centrifuged.

Laboratory measurements and the triptorelin stimulating test

The blood samples were taken in the morning during a fasting state. Growth hormone (GH), insulin-like growth factor-1 (IGF-1), serum-free T3, serum-free T4, thyroid-stimulating hormone (TSH), prolactin (PRL), serum total cortisol (at 8:00 a.m.), and adrenocorticotropic hormone (ACTH, at 8:00 a.m.) were measured via chemiluminescent methods. FSH, LH, and total testosterone were measured with commercial chemiluminescence kits (ACS: 180 Automatic Chemiluminescence Systems, Bayer, Germany). The intra- and interassay variation coefficients were 3.9% and 4.5% for FSH, 2.3% and 2.8% for LH, and 5.6% and 6.6% for total testosterone, respectively. The lowest measurable limits were 0.23 IU l^{-1}, 0.07 IU l^{-1}, and 5.2 ng dl^{-1} for FSH, LH, and total testosterone, respectively.

For patients on GnRH treatment, the blood samples for measuring FSH, LH, and testosterone were taken in the morning 30 min after a GnRH pulse was automatically infused. The mean value of FSH, LH, and testosterone obtained during the final two consecutive clinic visits were used as "values after pulsatile GnRH treatment" for statistical analysis.

For each patient, a GnRH analog (triptorelin) stimulating test was conducted to evaluate the gonadotropin reservoir in the pituitary gland. Triptorelin (100 μg) was injected intramuscularly, and serum LH and FSH were measured immediately and 60 min after injection. Previous data showed that 90% of CHH patients had stimulated serum LH_{60min} lower than 4 IU l^{-1}.[9]

Statistical analyses

SPSS version 17.0 (IBM, New York, NY, USA) was used for all data analyses. Normally distributed data were expressed as the mean ± s.d., and nonnormally distributed data were expressed as the median (quartiles). Comparisons between subgroups (Kallmann syndrome or not; cryptorchidism or not; poor or normal LH response subgroups) were conducted by unpaired t-tests. Predictors for pituitary response to GnRH therapy were evaluated by linear Cox regression analysis. The possible predictors obtained from the published studies,[4,10] such as basal levels of gonadotropins and testosterone, cryptorchidism, testicular size, and diagnosis (Kallmann syndrome or nCHH), were included into the model. Statistical significance was set at $P < 0.05$.

RESULTS

Clinical characteristics of CHH patients

In total, 82 CHH patients aged 24.3 ± 5.9 years, receiving GnRH therapy for at least 1 month, were included for statistical analyses. The median time of GnRH therapy was 4 months (range: 1–26 months). Patients were generally in good health with normal routine blood and urine test results and normal liver and renal function.

Pulsatile gonadorelin was administered subcutaneously at 10 μg per 90 min (approximately 133 ng kg^{-1}). During the treatment, GnRH dose was adjusted to achieve and maintain LH and FSH at the range of 5–10 IU l^{-1}. The final average GnRH dosage was 9.9 ± 0.4 μg per 90 min. The dose of GnRH was increased for nine patients and decreased for 26 patients. The remaining 47 patients continued with the starting dose.

Hormone response to GnRH therapy

In general, LH increased from 0.4 ± 0.5 IU l^{-1} to 7.5 ± 4.4 IU l^{-1} and FSH increased from 1.1 ± 0.9 IU l^{-1} to 8.8 ± 5.3 IU l^{-1} after treatment for a median of 4 months (range: 1–26 months); serum total testosterone increased from 29 ± 22 ng dl^{-1} to 224 ± 165 ng dl^{-1}; testicular size enlarged from 2.4 ± 1.6 ml to 7.5 ± 4.5 ml; and LH and FSH gradually increased during the therapy and plateaued after treatment for 1 month (**Figure 1**). During therapy, the gonadotropin levels at 1 month were higher than that at 1 week. Serum testosterone gradually increased for 2–3 months after treatment. The rate of successful spermatogenesis was 32.9% (27/82).

During the treatment, the increasing LH was linearly and positively associated with simultaneous FSH level (Pearson's correlation coefficient: 0.52, P = 0.000). After GnRH treatment for 1 month, 9 (11.0%) out of 82 patients still had <2 IU l^{-1} LH levels and 4 (4.9%) out of 82 patients had LH levels between 2 IU l^{-1} and 3 IU l^{-1}. 5 (6.1%) out of 82 patients had FSH levels below 2 IU l^{-1}, and 2 (2.4%) out of 82 patients had FSH levels between 2 IU l^{-1} and 3 IU l^{-1}. If taking "LH <2 IU l^{-1} or FSH <3 IU l^{-1}" as a cut point of normal pituitary response, 11 (13.4%) out of 82 patients would be classified into a "poor response subgroup": 5 (6.1%) out of 82 patients had low levels of both LH and FSH, 4 (4.9%) out of 82 patients had low levels of LH alone, and 2 (2.4%) out of 82 had low levels of FSH alone. If taking "LH <2 IU l^{-1}" as a cut point of normal pituitary response instead, 9 (11.0%) out of 82 patients would be classified into a low response subgroup.

Subgroup analysis

Patients were divided into Kallmann (n = 46) or nCHH (n = 36) subgroups according to their olfactory status. At baseline, basal LH level was 0.3 ± 0.4 IU l^{-1} versus 0.6 ± 0.5 IU l^{-1} (P = 0.006) in the Kallmann and nCHH subgroups, respectively. The LH_{60min} level (after triptorelin stimulation) was 4.5 ± 6.2 IU l^{-1} versus 8.4 ± 9.2 IU l^{-1} (P = 0.03) in Kallmann and nCHH subgroups, respectively.

Patients were classified into cryptorchidism (n = 13) or noncryptorchidism (n = 69) subgroups. At baseline, the LH level was 0.2 ± 0.5 IU l^{-1} versus 0.4 ± 0.5 IU l^{-1} (P = 0.117) between the two subgroups. LH_{60min} level (after triptorelin stimulation) was 4.8 ± 9.2 IU l^{-1} versus 6.4 ± 7.8 IU l^{-1} (P = 0.541) between the two subgroups, and after 1 month of GnRH treatment, the LH level increased to 7.5 ± 4.6 IU l^{-1} versus 7.5 ± 4.4 IU l^{-1} (P = 0.879) in the two subgroups.

Patients were divided into normal (n = 73) or poor (n = 9) LH response subgroups according to their LH levels (<2 IU l^{-1} or ≥2 IU l^{-1}) at 1 month of GnRH therapy (**Table 1**).

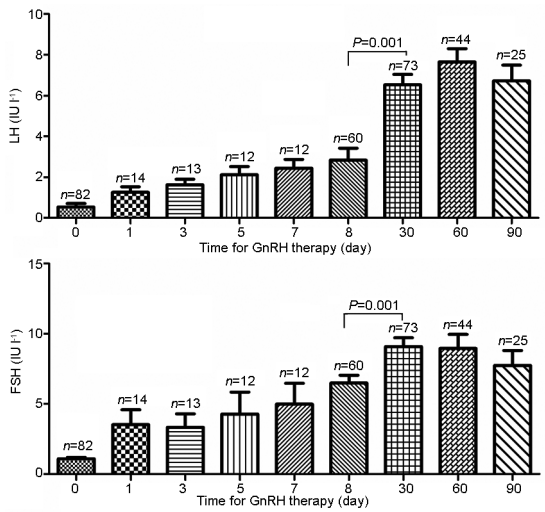

Figure 1: LH and FSH levels gradually increase during pulsatile GnRH therapy. After 1 month of treatment, FSH and LH levels maintained at a stable level. GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone.

In the normal LH response subgroup, after GnRH therapy for a median of 5 months (range: 1–26 months), the LH and FSH levels increased to 8.3 ± 4.0 IU l^{-1} and 9.4 ± 5.2 IU l^{-1}, respectively. In the poor LH response subgroup, after GnRH therapy for a median of 2 months (range: 1–6 months), the levels of LH and FSH increased to 1.0 ± 0.5 IU l^{-1} and 3.7 ± 3.2 IU l^{-1}, respectively. Accordingly, the normal LH response subgroup had higher testosterone levels (248 ± 158 ng dl^{-1} *vs* 39 ± 28 ng dl^{-1}, P = 0.001, respectively) and a larger testicular size (7.9 ± 4.5 ml *vs* 4.0 ± 3.1 ml, P = 0.005, respectively) than the poor LH response subgroup. The final GnRH dosage in the normal and poor response subgroups was 9.4 ± 0.4 μg versus 14 ± 0.8 μg per 90 min (equivalent to 125 ± 5 ng kg^{-1} *vs* 187 ± 11 ng kg^{-1}), P = 0.049. There were 11 and 2 patients with cryptorchidism, respectively, in the normal and poor response subgroups.

Predictors for LH response to pulsatile GnRH therapy

Possible predictive factors for LH response to GnRH therapy, such as diagnosis (Kallmann or nCHH), cryptorchidism history, basal testicular size, basal and triptorelin-stimulated gonadotropin levels, and other factors (**Table 2**), were included in a linear Cox regression analysis. Higher levels of basal total serum testosterone (β = 0.252, P = 0.029) and triptorelin-stimulated FSH (FSH_{60min}; β = 0.518, P = 0.010) both favorably predicted a normal LH response to pulsatile GnRH therapy.

Further analysis (Pearson's relationship) showed that both FSH_{60min} (coefficient 0.3, P = 0.007) and basal testosterone (coefficient 0.235, P = 0.033) were independently and positively associated with LH levels after GnRH treatment (**Figure 2**).

DISCUSSION

The present study revealed that 11.0% of CHH patients had a poor response to pulsatile GnRH treatment, defined as LH <2 IU l^{-1} after GnRH treatment for 1 month. Higher levels of baseline testosterone and triptorelin-stimulated FSH_{60min} each favorably predicted the pituitary response to GnRH therapy.

Pulsatile GnRH was effective at inducing spermatogenesis in most patients with CHH.[3,4,11] One recent study that included ninety CHH patients treated with pulsatile GnRH showed that 10% of patients had a poor response to GnRH therapy, defined as "inappropriately low LH levels after high dosage of GnRH (up to 800 ng kg^{-1} per pulse)."[4]

Table 1: Difference between subgroups with poor and normal pituitary response to pulsatile gonadotropin-releasing hormone therapy

	All CHH patients (n=82)	*Poor LH response subgroup (n=9)*	*Normal LH response subgroup (n=73)*	*P**
Baseline				
Basal LH (IU l^{-1}), mean±s.d.	0.4±0.5	0.3±0.3	0.4±0.4	0.135
Basal FSH (IU l^{-1}), mean±s.d.	1.1±0.9	0.8±0.8	1.1±1.0	0.572
Basal testosterone (ng dl^{-1}), mean±s.d.	29±22	17.2±9.7	32.3±28.7	0.108
Basal testicular size (ml), mean±s.d.	2.4±1.6	2.0±1.1	2.5±1.7	0.410
Cryptorchidism history, *n* (%)	13 (15.9)	2 (22.2)	11 (15.1)	0.785
After pulsatile GnRH therapy				
Follow-up period (month), median (range)	4 (1–26)	2 (1–6)	5 (1–26)	0.041
Final GnRH dosage (μg per 90 min), mean±s.d.	9.9±0.4	14±0.8	9.4±0.4	0.049
LH (IU l^{-1})	7.5±4.4	1.0±0.5	8.3±4.0	0.001
FSH (IU l^{-1})	8.8±5.3	3.7±3.2	9.4±5.2	0.001
Testosterone (ng dl^{-1})	224±165	39±28	248±158	0.001
Testicular size (ml)	7.5±4.5	4.0±3.1	7.9±4.5	0.005
Success rate of spermatogenesis (%)	33	0	37	0.014
Pregnancy (*n*)	4	0	4	0.028

*Comparison between poor and normal LH response subgroups. CHH: congenital hypogonadotropic hypogonadism; GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone; s.d.: standard deviation

Table 2: Possible predictors for pituitary response to gonadotropin-releasing hormone therapy

	Unstandardized coefficients		*Standardized coefficients*	*t*	*P*
	B	*s.e.*	*β*		
Diagnosis (Kallmann, nCHH)	–2.006	1.061	–0.224	–1.891	0.063
Basal testicular size	0.151	0.340	0.056	0.444	0.658
Cryptorchidism	0.598	1.436	0.047	0.416	0.679
Basal LH	0.999	1.489	0.104	0.671	0.505
Basal FSH	–0.699	0.747	–0.153	–0.936	0.353
LH_{60min}*	–0.144	0.103	–0.258	–1.399	0.166
FSH_{60min}*	0.849	0.320	0.518	2.652	0.010
Basal testosterone	0.050	0.022	0.252	2.229	0.029

Dependent variable: LH levels after pulsatile GnRH therapy. *The level of gonadotropin at 60 min in triptorelin-stimulating test. nCHH: normosmic congenital hypogonadotropic hypogonadism; GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone; s.e.: standard error

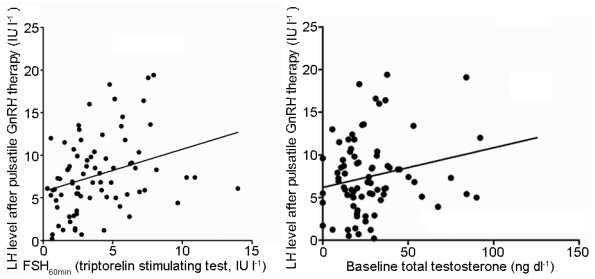

Figure 2: Baseline testosterone and triptorelin-stimulated FSH_{60min} levels are positively associated with LH level after pulsatile GnRH therapy. GnRH: gonadotropin-releasing hormone; LH: luteinizing hormone; FSH: follicle-stimulating hormone.

Another study found that 97% of CHH patients "normalized" their LH level after GnRH therapy for 1 year, with a dosage of GnRH increasing up to 600 ng kg^{-1}.[11] A pituitary resistance to pulsatile GnRH therapy also manifested in 30% of female CHH patients.[12] The current data are consistent with these other studies and show that 11% of CHH patients had a poor pituitary response even when the dosage of GnRH was adjusted to as high as 190 ng kg^{-1} per pulse.

The cutoff value for "normal pituitary response to GnRH therapy" is difficult to define. Some studies have taken LH as the primary biomarker for gonadotrophic response to GnRH stimulation.[13] Our data demonstrate that the increase in LH is linearly and positively associated with FSH after GnRH therapy. Given this pattern, it is possible–and likely more convenient–to evaluate the pituitary response by LH level alone.

The levels of gonadotropins gradually increased across the 1st week of pulsatile GnRH treatment, consistent with a previous clinical study.[14] In their study, a greater LH response was achieved in partial CHH patients than complete CHH patients during the 1st week of GnRH treatment.[14] In clinical practice, if patients' gonadotropins did not remarkably increase after 7 days of GnRH therapy, they would be considered as "failure to GnRH therapy."[15] However, our study shows that the levels of gonadotropins are higher after 1 month of GnRH treatment than after 1 week of treatment. Therefore, the timeline for evaluating pituitary response to GnRH therapy should be delayed to at least 1 month after the start of GnRH treatment.

Several mechanisms could underlie a poor gonadotropin response to GnRH therapy. First, patients whose CHH status is caused by a mutation in the GnRH receptor that renders it inactive may be unable to respond to GnRH therapy.[12,16] Second, other CHH-causing genes, including *KAL1* and *FGFR1*, are expressed in both the pituitary gland and the testes,[12] and both have been associated with pituitary resistance to GnRH therapy.[4]

Linear Cox regression analysis showed that higher levels of baseline testosterone and stimulated FSH_{60min} were favorable predictors of the pituitary response to GnRH therapy. The results are not unexpected because these two measures reflect the reservoir of gonadotrophs in the pituitary. Previous studies showed that larger testicular size was associated with a greater LH response to GnRH therapy,[14] and that baseline gonadotropins and testicular size were important predictors for sperm induction[3,10,11] in CHH patients. However, in our analysis, they were not associated with pituitary response to GnRH therapy. Instead, our results suggest that baseline testosterone and stimulated FSH_{60min} would serve as better predictors of the patient's potential response to GnRH therapy.

There are some limitations to this study that should be addressed. First, the GnRH dosage was approximately 14 μg per pulse (equal to 187 ng kg^{-1}) in the poor response subgroup, over two times the physiologic dose of 75 ng kg^{-1}.[12] It is possible that higher doses of GnRH (up to 600–800 ng kg^{-1}) may induce more gonadotropin secretion.[10,17] Second, the follow-up time was too short to fully clarify the therapeutic effects of treatment on spermatogenesis for patients with a normal pituitary response. Some of those pituitary responders still may not achieve appropriate spermatogenesis due to testicular resistance to gonadotropins.[4,18] Third, 2 IU l^{-1}, the LH cutoff value in our study, is the lower limit for normal adult men. This cutoff point may not be appropriate for CHH patients, for this patient population may require higher levels of gonadotropin to stimulate sperm production. Last, the CHH-related genes were not screened in this population. Possible mutations in *KAL1*, *FGFR1,* and *DAX1* may cause pituitary defects and failed pituitary response to GnRH therapy.[4,14]

CONCLUSION

Our study supports the consensus that pituitary resistance occurs in 11.0% of CHH patients, who have a poor LH response to GnRH therapy. It also suggests that higher levels of basal testosterone and triptorelin-stimulated FSH_{60min} are favorable predictors for pituitary response to GnRH therapy.

AUTHOR CONTRIBUTIONS

JFM, XW, and JJZ collected clinical data. ZXL, HLX and BKH performed patient follow-up and collected clinical data. XYW and MN designed the study and edited the article. All authors read and approved the final manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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