

The Role of Single Nucleotide Polymorphisms in Transporter Proteins and the Folate Metabolism Pathway in Delayed Methotrexate Excretion: A Case Report and Literature Review

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Abstract: High-dose methotrexate (HDMTX) is a pivotal component of the chemotherapeutic regimens of osteosarcoma. However, the use of HDMTX is limited by an increased risk of dose-dependent toxicity. It is thought that the plasma levels and therapy-related toxicity of MTX could be associated with single nucleotide polymorphisms (SNPs) within MTX metabolism pathway genes. Here, we report a case of a paediatric osteosarcoma girl with delayed MTX excretion who was successfully managed using supportive measures and continuous veno-venous haemodiafiltration. We further identified the cause that could account for delayed elimination by genotyping analysis. The results showed that variations have been found in *SLCO1B1*, *SLC19A1*, *ABCB1* and *MTHFR*, all those were reported to have a strong association with delayed elimination of MTX in clinical studies. After comprehensive consideration of genotype and clinical phenotype, the second course of HDMTX was administered to this patient at a half reduced dose. We also performed a literature review to summarize the pharmacogenetic factors that influence HDMTX pharmacokinetics or MTX-related adverse effects in osteosarcoma patients. It is suggested that the potential risk of delayed MTX elimination is worthy of clinical attention, and the implementation of genotyping should be considered to ensure therapeutic safety.

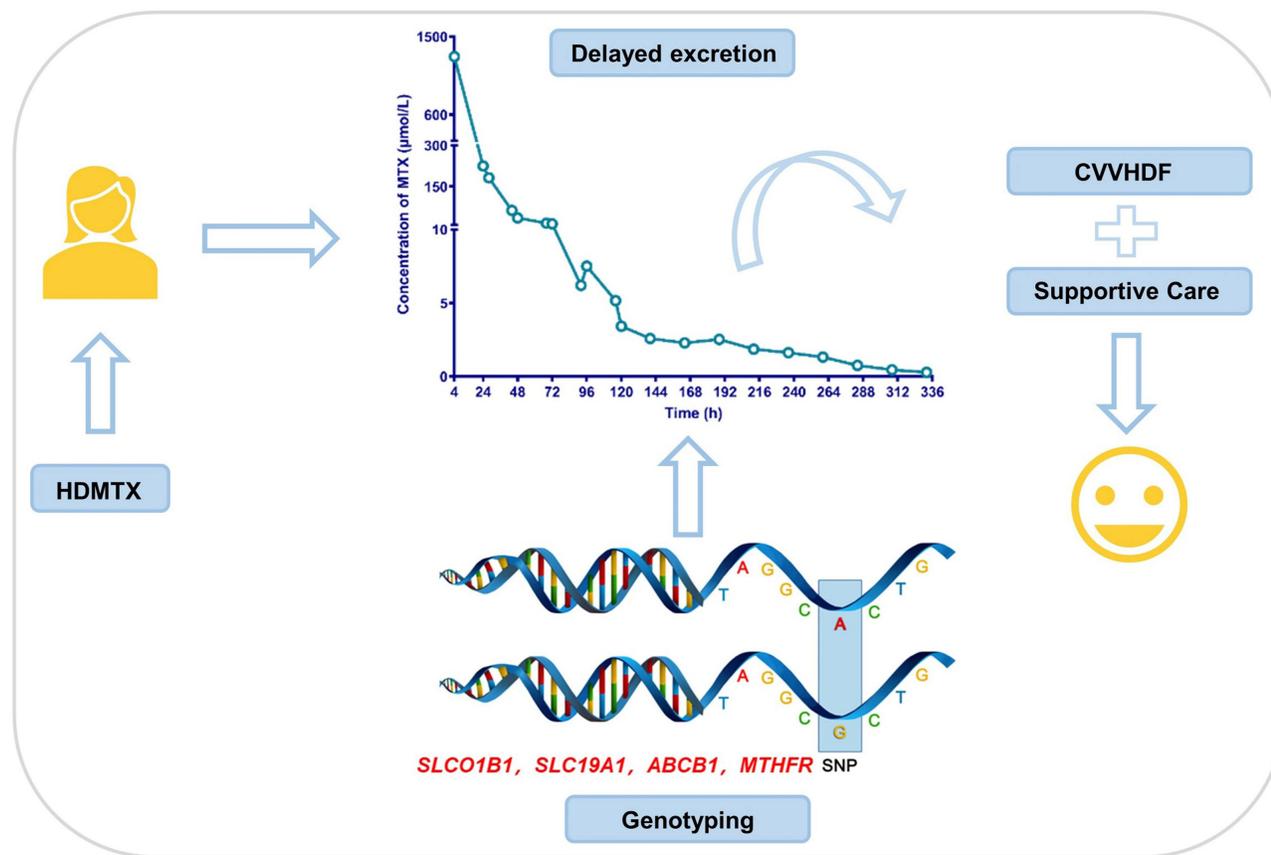
Keywords: high-dose methotrexate, osteosarcoma, delayed excretion, single nucleotide polymorphisms

Introduction

Osteosarcoma (OS) is the most common malignant bone tumour and mostly affects teenagers.¹ High-dose methotrexate (HDMTX) combined with doxorubicin and cisplatin is the “gold standard” chemotherapy for osteosarcoma.² Unfortunately, because the dose of HDMTX (8–12 g/m²) is dozens of times more than the common dose, patients receiving this strategy often suffer from significant toxicities of the kidneys, the liver and gastrointestinal tract as well.³ Therefore, supportive measures, including folate supplementation, fluid hyperhydration, and urine alkalization before and during MTX treatment, are used to facilitate renal elimination to reduce HDMTX-related toxicities.⁴ Nevertheless, there is still considerable interpatient variability in the clearance of HDMTX, with severe delayed MTX excretion seen from 0.86 to 1.8% in youths with OS.⁵ Factors including age, hydration status and the concurrent use of nephrotoxic agents are shown to influence MTX clearance.⁶ Furthermore, recent pharmacogenetic studies have investigated the effects of several genes and polymorphisms on MTX clearance,^{7,8} which may help us to better understand the pharmacokinetic variability and improve patient outcomes.

Here, we report a case of paediatric osteosarcoma in whom delayed MTX excretion was successfully managed using continuous veno-venous haemodiafiltration (CVVHDF) and supportive measures. Most importantly, polymorphisms in genes encoding transporter proteins or folate pathway genes partially account for delayed MTX excretion. According to

Graphical Abstract



the pharmacogenetic results, a reduced MTX dose (6 g/m^2) was optimized for the second course of chemotherapy and completed uneventfully.

Case Summary

A 12-year-old girl was diagnosed with osteosarcoma of the right proximal femur and received chemotherapy in our department of haematology and oncology. Her laboratory test results, including the indicators of liver and kidney function, were all in the normal range. Her first course of HDMTX was administered at 12 g/m^2 . Six hours before MTX infusion, she was intravenously hydrated with 500–1000 mL/m² of 5% glucose together with 5 mL/kg of 5% NaHCO₃ solution. MTX was dissolved in 500 mL of D5W and infused over 4 hours. After this MTX infusion, she was hydrated with the same fluid to a total of 3000–4000 mL/m² per day during the first 48 hours. Her 4-hour MTX level was 1270.08 µmol/L. Her 24-hour and 28-hour MTX levels were 224.64 and 181.4 µmol/L, respectively (Figure 1A). Thus, leucovorin was immediately escalated to 200 mg/m² and administered every 6 hours. The girl developed acute kidney injury (AKI) with an increasing serum creatinine level of 197 µmol/L (Figure 1B). She also suffered from acute liver failure with prothrombin activity less than 40%, an increased ALT and AST levels (1247 U/L and 823 U/L, respectively, Figure 1B). She was recommended to be transferred to pediatric intensive care unit (PICU) for CVVHDF management. Furthermore, repeated blood routine tests showed that the number of white blood cells, the proportion of neutrophils and the PCT levels were significantly higher than normal. The patient developed a fever, so bacteremia was considered, and meropenem was used to protect against infection. During CVVHDF, omeprazole was given to protect the stomach, atomolan and compound glycyrrhizin were administered to protect the liver, and leucovorin rescue was continued at

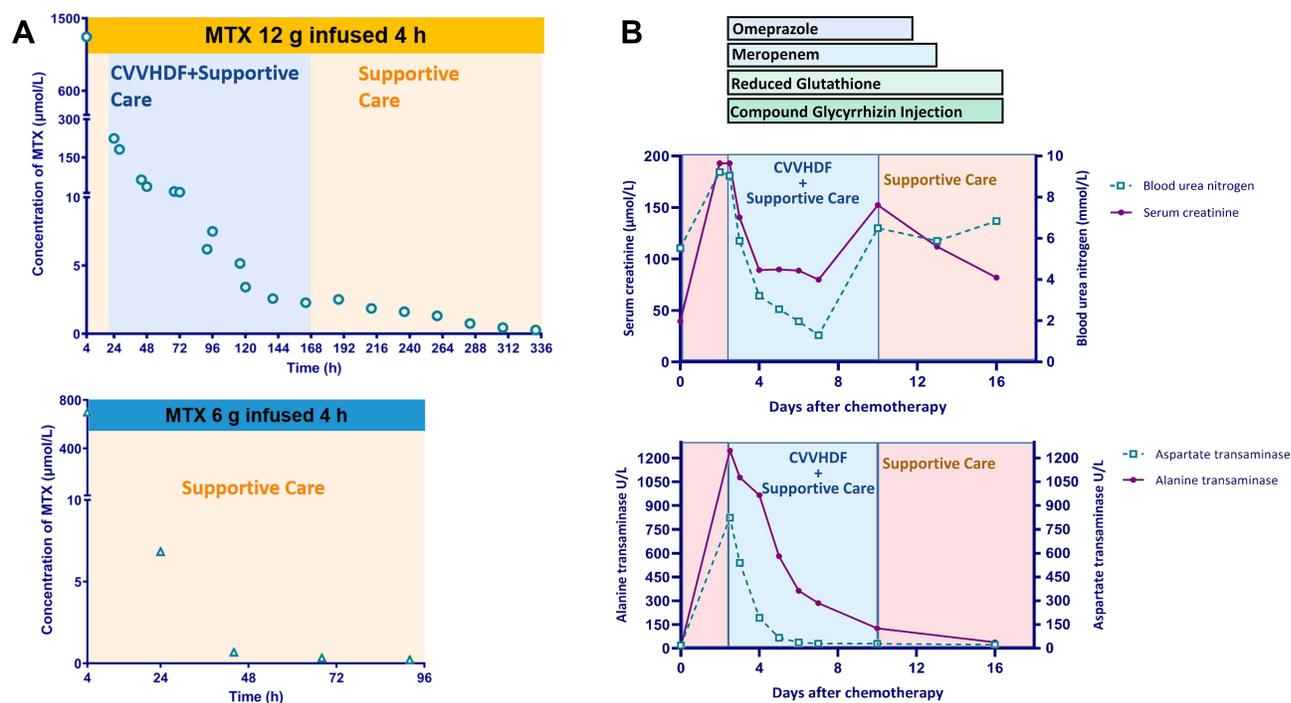


Figure 1 Time course of plasma methotrexate concentrations (**A**) and medications and liver/renal function (**B**).

a dose that was administered according to the MTX concentration. After a total of 5 days for CVVHDF, serum ALT and AST levels were decreased to 285 U/L and 30 U/L, respectively, and the serum creatinine level was 79.9 $\mu\text{mol/L}$ (Figure 1B). The MTX concentration was decreased to 2.28 $\mu\text{mol/L}$. Then the patient was transferred back to the general ward for further treatment. Over the following hospital days, her MTX level decreased very slowly and finally reached to 0.28 $\mu\text{mol/L}$ after 14 days of high-dose leucovorin in rescue (Figure 1A). Another two days later, indicators of liver and kidney function returned to normal.

To identify the cause that could account for delayed elimination and to explore a suitable dosage strategy for the next chemotherapy of MTX, we selected the following candidate genetic variants, rs1051296 in *SLC19A1*; rs4149056 and rs11045879 in *SLCO1B1*; rs1045642, rs2032582 and rs1128503 in *ABCB1*; rs717620 in *ABCC2*; rs1801131 and rs1801133 in *MTHFR*, for genotyping by real-time PCR. As shown in Table 1, variations have been found in *SLCO1B1*, *SLC19A1*, *ABCB1* and *MTHFR*, all of which were reported to have a strong association with delayed elimination of MTX in clinical studies.

After comprehensive consideration of genotype and clinical phenotype, the second course of HDMTX was administered to this patient at a half reduced dose (6 g/m^2). The 4-hour and 24-hour MTX levels were 699.84 and 6.84 $\mu\text{mol/L}$, respectively. Continuous leucovorin rescue was performed according to the MTX concentration. After 3 days, the MTX level was decreased to 0.22 $\mu\text{mol/L}$ (Figure 1A). Following recovery, she proceeded to chemotherapy comprising of cisplatin (60 $\text{mg/m}^2 \cdot \text{d}$) and epirubicin (37.5 $\text{mg/m}^2 \cdot \text{d}$) and completed the course uneventfully.

Discussion

Although HDMTX (8–12 g/m^2) has been shown to be the most effective single agent in the neoadjuvant therapy of OS, delayed excretion of MTX can result in life-threatening toxicity that may lead to treatment cessation, irreversible organ damage, and even death. As it excreted primarily by renal route, the precipitation of MTX or its relatively insoluble metabolites in the renal tubules might be one of the most likely mechanisms for acute nephrotoxicity and then result in delayed excretion.⁹ More important, delayed MTX excretion may occur in the absence of any predisposing factor.⁶ In this case, the girl suffered from “severe” delayed excretion ($C_{24\text{h}} > 50 \mu\text{mol/L}$) and acute liver and kidney injury. She was successfully managed using supportive cares and CVVHDF.

Table 1 Genotype of the Targeted SNPs

Gene	rs No.	SNP	Genotype
<i>SLCO1B1</i>	rs4149056	TT	wt
	rs11045879	CT	het
<i>SLC19A1</i>	rs1051296	AC	het
<i>ABCB1</i>	rs1045642	AG	het
	rs2032582	AC	het
	rs1128503	AA	wt
<i>ABCC2</i>	rs717620	CC	wt
<i>MTHFR</i>	rs1801133	CT	het
	rs1801131	TT	wt

Note: The SNPs in bold type indicate mutations.

Abbreviations: wt, homozygous wild-type genotype; het, heterozygous variant genotype.

The pharmacokinetics of HDMTX showed large individual variability. A reduced effective blood volume or the concurrent use of nephrotoxic agents were known to be related to the development of delayed MTX excretion.¹⁰ However, the patient had no signs of decreased blood volume and did not receive any nephrotoxic agent. Before administration, her renal function was normal, and her hydration status was standard. The reason for delayed MTX excretion is worth exploring. Recently, growing evidence suggested that genetic variants of metabolic enzymes^{11,12} or transporters^{13,14} involved in MTX elimination could provide a mechanistic explanation for the variability of its pharmacokinetics. Furthermore, these variants might be potential predictors for personalized pharmacotherapy. Therefore, we focused on the pharmacogenomics of MTX to illustrate the causes of delayed MTX elimination.

Generally, pharmacogenetic studies have identified various genes that contribute to the vast interindividual variation in MTX pharmacokinetics and are mainly involved in transporter genes (*SLCO1B1*, *SLC19A1*, *ABCB1*, and *ABCC2*) or folate pathway genes (*MTHFR*, *MTR*, *MTRR*, and *DHFR*). However, those studies that focused on OS patients were very limited. We first searched PubMed with a combination of the keywords “pharmacogenetic” AND “methotrexate” AND “osteosarcoma”; the information of these studies is summarized and presented in Table 2. Of those genes, the SNPs on *ABCG2*,⁷ *ABCB1*, *ABCC3*,¹⁵ and *SLCO1B1*¹⁶ were associated with MTX-PK parameters. Other genetic variants, such as *MTHFR* (rs1801133), *RFC1* (rs1051266), *GSTP1* (rs1695), and *MTR* (rs1805087) were shown to be associated with an increased risk of HDMTX-related adverse effects.^{17–23} In addition, rs4148416 in *ABCC3* and three SNPs in *ABCB1*, rs4148737, rs1128503 and rs10276036 were associated with overall survival.²⁴

Interestingly, *SLCO1B1* was considered to be the only gene that reliably demonstrates an effect on MTX pharmacokinetics in a recent systematic review.²⁵ Notably, rs4149081 and rs11045879 in *SLCO1B1* have been strongly associated for the first time with MTX clearance.^{26,27} The rs11045879 variant was found in this patient, which may partially account for the delayed excretion. However, one study showed variants in *SLCO1B1* explained up to 10% of the interpatient variability in the clearance of HDMTX.²⁶ Hence, other MTX transporters could be of interest. Among them, the most studied was *SLC19A1*. Overall, *SLC19A1* is an important transporter responsible for folate homeostasis and the uptake of endogenous reduced folates and anti-folate xenobiotics including MTX.²⁸ In Chinese children with acute lymphoblastic leukaemia, delayed elimination of MTX was more frequent in rs1051296 mutant carriers than in wild-type patients.²⁹ This girl carried a heterozygous variant of *SLC19A1*, increasing the risk of delayed elimination. On the other hand, pumping out MTX from the cell was mediated by highly polymorphic ABC (specifically the *ABCB1*, *ABCC2*, *ABCC4* and *ABCG2*) transporters.³⁰ Polymorphisms in these genes have been studied in early studies, however, the results are inconsistent.^{7,21} This girl has two heterozygous mutations in rs1045642 and rs2032582 in *ABCB1*, which might lead to a decrease in MTX clearance.⁸

Table 2 Pharmacogenetic Literatures Information of HD-MTX in OS

First Author and Year	Study Population	Principal Findings	References
Lui G, (2018)	OS2006 trial (a French multicentre, open-label, parallel group, Phase III, randomized controlled trial)	Rs13120400, rs13137622 and rs12505410 on <i>ABCG2</i> gene and rs4148324 in <i>UGT1A</i> gene were significantly associated with MTX-PK CL.	[7]
Hegy M, (2017)	59 children Hungarian diagnosed with OS	The SNPs <i>ABCB1</i> rs928256, <i>ABCC3</i> rs4793665, <i>GGH</i> rs3758149, and <i>NR1I2</i> rs3814058 SNPs were associated with MTX-PK parameters.	[15]
Lambrecht L, (2017)	48 Belgium patients of new diagnoses with OS <18 years	The <i>MTHFR</i> C667T (rs18011330) polymorphism is associated with toxicity or overall survival, but could be used for relapse risk stratification	[17]
Claudia M, (2016)	196 patients with newly diagnosed HGOS in Italy	Genotypes of <i>ABCC2</i> (rs2273697), <i>GH</i> (rs11545078), <i>TP53</i> (rs1642785), <i>CYP2B6</i> *6 (rs3745274 and rs2279343) were associated with EFS. Genotypes of <i>ABCB1</i> (rs1128503), <i>ABCC2</i> (rs2273697), <i>ABCC2</i> (rs3740066), <i>ERCC1</i> (rs3212986), <i>XPB</i> (rs1799793), <i>XRCC3</i> (rs861539), <i>MTHFR</i> (rs1801131) and <i>GGH</i> (rs1800909) were associated with elevated risk for toxicity development.	[18]
S Jabeen, (2015)	62 HDMTX-treated OS patients at The Norwegian Radium Hospital	<i>RFC1</i> (rs1051266) had significantly better survival and a lower frequency of metastasis. Rs1053129 in the dihydrofolate reductase (<i>DHFR</i>) gene were more likely to have a metastasis.	[19]
Goričar K, (2014)	118 OS patients were diagnosed in Slovenia	Polymorphic <i>SLCO1B1</i> rs4149056 and rs11045879 alleles were associated with significantly higher serum methotrexate area under the curve.	[16]
MM Hagleitner (2014)	115 Dutch paediatric oncology patients. These include 63 newly diagnosed, high-grade OS patients and 52 patients with denovo acute lymphoblastic leukaemia (ALL).	The <i>MTHFR</i> 677T allele (rs1801133) has only a minor role in the development of MTX-induced hepatotoxicity.	[20]
Rachael E, (2012)	60 patients who had completed MAP chemotherapy	<i>ABCC2</i> (rs717620) and <i>GSTP1</i> (rs1695) were associated with poor histological response, whereas <i>MTHFD1</i> (rs2236225) was protective. Methotrexate toxicity was increased in variants of <i>MTHFR</i> (rs1801131), <i>ABCB1</i> (rs1045642), and <i>ABCC2</i> (rs17222723). Variants of <i>GSTP1</i> (rs1695) were at increased risk of myelosuppression and cardiac damage.	[21]
Caronia D, (2011)	102 consecutive patients diagnosed with OS at the University Clinic of Navarra, Pamplona, Spain	Rs4148416 in <i>ABCC3</i> , and three SNPs in <i>ABCB1</i> , rs4148737, rs1128503 and rs10276036 were associated with overall survival.	[24]
Ana Patino-Garci, (2009)	96 children and adolescents with osteosarcoma	<i>MTHFR</i> rs1801133 and <i>MTR</i> rs1805087 were associated with haematologic and gastrointestinal toxicity.	[22]
Judit Muller, (2008)	A 10-year-old boy with OSC	The authors hypothesize that MTX toxicity was associated between homozygosity of the <i>MTHFR</i> rs1801133 polymorphism.	[23]

In addition, the girl carried a T variant of rs1801133 in *MTHFR*. *MTHFR* is responsible for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. It has been confirmed that reduced *MTHFR* expression is related to MTX-induced toxicity in patients with leukaemia.³¹ Furthermore, both SNPs of *MTHFR* C677T (rs1801133) and *MTHFR* A1298C (rs1801131) have been suggested to be accompanied by decreased enzyme activity.³² Some studies found that the T allele in rs1801133 was associated with decreased elimination of MTX, while few studies found a clinically significant association between MTX elimination and rs1801131.^{33,34} Therefore, we hypothesized that carrying with the T allele may aggravate the plasma MTX level and increase the risk of adverse effects in this case.

Based on the genotype results of this patient and previous literatures,³⁵ we speculate that genetic polymorphisms in the transporters and folate pathways at least partially account for delayed MTX elimination. We consider that implementation of genotyping before HDMTX chemotherapy may help predict MTX treatment response. However, it is worth noting that the pharmacogenetics of MTX are still at the bench level. There is a lack of a definite quantified association between each genetic polymorphism and the efficacy or adverse effects of HDMTX. Therefore, only one pharmacogenetic variant alone may not have sufficient predictive power. A panel consisting of multiple genes should be considered in the clinic.

Here, we describe a valuable case of delayed MTX elimination, possibly attributed to genetic mutations in transporters (*SLCO1B1*, *SLC19A1* and *ABCB1*) and the folate pathway (*MTHFR*). The potential risk of delayed MTX elimination is worthy of clinical attention, and the implementation of genotyping should be considered to ensure therapeutic safety.

Abbreviations

HDMTX, high-dose methotrexate; MTX, methotrexate; SNPs, single nucleotide polymorphisms; *SLCO1B1*, solute carrier organic anion transporter family member 1B1; *SLC19A1*, solute carrier family 19 member 1; *ABCB1*, ATP binding cassette subfamily B member 1; *MTHFR*, methylenetetrahydrofolate reductase; OS, osteosarcoma; CVVHDF, continuous veno-venous haemodiafiltration; D5W, dextrose 5% water solution; AKI, acute kidney injury; ALT, alanine aminotransferase; AST, aspartate aminotransferase; PICU, pediatric intensive care unit; PCT, procalcitonin; PCR, polymerase chain reaction; *ABCC2*, ATP binding cassette subfamily C member 2; *MTR*, 5-methyltetrahydrofolate-homocysteine methyltransferase; *MTRR*, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase; *DHFR*, dihydrofolate reductase; *ABCG2*, ATP binding cassette subfamily G member 2; *ABCC3*, ATP binding cassette subfamily C member 3; PK, pharmacokinetics; *RFC1*, reduced folate carrier protein 1; *GSTP1*, glutathione S-transferase pi 1; *ABC*, ATP binding cassette family; *ABCC4*, ATP binding cassette subfamily C member 4.

Patient Consent Statement

Written informed consent was obtained from the patient's mother for the publication of this case report. The study was approved by the Ethics Committee of Children's Hospital of Nanjing Medical University (202203007-1).

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Disclosure

Yue-Tao Zhao is a visiting graduate student from China Pharmaceutical University. The authors declare no other conflicts of interest in this work.

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