Original Article

Randomized trial of FX high flux vs standard high flux dialysis for homocysteine clearance

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Abstract

Background. Cardiovascular disease is the major cause of death in the end-stage renal disease population. Novel risk factors such as homocysteine (Hcy) are of considerable interest in this group as hyperhomocysteinaemia is highly prevalent in the setting of renal impairment. Folic acid–vitamin B group therapies are only partially effective treatments. Hcy is highly protein-bound and thus poorly dialysed. Dialyzers with albumin-leaking properties have been shown to result in lowering of plasma Hcy. As the FX-class (Advanced Fresenius Polysulfone dialyzer) has greater clearance of larger molecular weight substances but is non-albumin-leaking, we explored the capacity of this new technology membrane to reduce plasma Hcy levels.

Methods. A prospective randomized cross-over trial in 35 prevalent haemodialysis patients, one group receiving 12 weeks dialysis using FX dialyzer then 12 weeks with standard high flux dialysis (SHF) and the other group SHF followed by FX dialyzer. All patients received vitamin B6 25 mg and folic acid 5 mg daily throughout the study.

Results. The primary outcome was plasma Hcy pre-dialysis at week 12. FX vs SHF showed no significant difference, 25±6.6 vs 25.9±5.8 µg/l, Δ95% CI = −2.77 to 4.59, P = 0.31. There was a non-significant trend toward a decrease in Hcy in both groups (27.43±7.68 to 25.91±5.78 µmol/l for SHF, P = 0.23 and 26.0±4.58 to 25.0±6.61 µmol/l for FX, P = 0.28). Analysis by repeated measures method demonstrated a statistically significantly lower Hcy with FX vs SHF dialyzer (adjusted β = −1.30, 95% CI = −2.41 to −0.19, P = 0.022). Kt/V urea was higher in FX vs SHF (1.35 ± 0.18 vs 1.22 ± 0.2; P = 0.013). Folate and B6 levels did not change.

Conclusions. The primary outcome analysis did not show any significant difference in pre-Hcy comparing FX and SHF membranes. Although our secondary analysis demonstrated a statistically significant difference between membranes, the magnitude of the difference (1.3 µmol/l) is not clinically significant. Thus the use of the FX dialyzer did not result in a clinically significant benefit in relation to improving pre-dialysis Hcy compared with standard high-flux dialysis.

Keywords: dialyzer membrane; dialysis; FX-class dialyzer; haemodialysis; high-flux; plasma homocysteine

Introduction

Cardiovascular disease (CVD) is highly prevalent in the end-stage renal disease (ESRD) population and is the predominant cause of death. The classical risk factors for cardiovascular disease do not appear to account entirely for the disease burden in this group of patients. A number of novel risk factors have been proposed, including homocysteine (Hcy), a sulphur-containing amino acid formed during methionine metabolism. There are data in the general population supporting the role of hyperhomocysteinaemia as a risk factor for CVD [1]. In the dialysis population the data are divergent, with some studies showing a relationship [2,3], and other studies showing an inverse relationship [4]. These observations are likely to be confounded by nutritional status.

There is considerable interest in therapies to normalize the elevation of Hcy seen in renal failure, and supplementation with folic acid, B12 and B6, which are cofactors in Hcy metabolism, has been partly
FX-class dialyzer and homocysteine clearance

effective but does not normalize Hcy in dialysis patients [5,6]. There has also been considerable interest in whether newer dialysis membranes may improve serum Hcy levels further. As Hcy is highly protein bound, it is not well cleared by conventional low-flux dialysis, when given in the standard ‘4 × 3’ format.

Enhancing the dialytic clearance of Hcy may represent an adjunctive therapeutic option. Several uncontrolled trials have been inconclusive in determining whether standard high-flux (SHF) dialysis provides an advantage over low-flux dialysis in relation to Hcy clearance [7–9], whereas a prospective, randomized study failed to demonstrate superiority of high-flux versus low-flux dialysis, as measured by pre-dialysis Hcy levels. This was despite high-flux treatment giving greater intradialytic clearances of Hcy, 42% with high-flux compared to 32% with low-flux, \( P < 0.001 \) [10].

One possible reason for the failure of high-flux dialysis to lower plasma Hcy levels may be binding of Hcy to albumin. There have been three publications utilizing highly permeable albumin-leaking membranes that have shown significant lowering of Hcy [11–13]. Van Tellingen et al. [11] and Galli et al. [13] both demonstrated a reduction in plasma Hcy of about 33% by 12 weeks, whereas De Vriese et al. [12] showed a fall in plasma Hcy of 14.6% at 4 weeks. The former two studies also confirmed the findings of previous investigations which showed that non-albumin-leaking high-flux membranes were not superior to low-flux membranes in relation to serum Hcy levels. Although clearance of protein-bound Hcy is partly responsible for improvement in plasma Hcy in the studies, the removal of large molecular weight inhibitors of Hcy metabolism has been proposed to be the predominant mechanism for the benefit seen.

There is some concern that albumin-leaking dialyzers could aggravate hypoalbuminaemia and malnutrition in ESRD patients [14], which could potentially out-weigh the cardiovascular risk benefit of Hcy lowering of such membranes. We hypothesized that a newly available polysulfone dialysis membrane developed specifically to increase clearance of larger uraemic toxins but being non-albumin-leaking (FX-class dialyzer, Fresenius Medical Care) may result in a fall in Hcy by improving metabolic clearance of inhibitors of Hcy without the concern of excess albumin loss. We therefore studied the effect of this new FX-class high-flux dialyzer compared to a standard high-flux dialyzer in relation to effect on pre-dialysis Hcy levels and intra-dialytic clearance of Hcy.

Subjects and methods

Study population

Patients from our haemodialysis program were invited to participate in the study if they were >18 years of age, were receiving standard 4–5 h three times per week dialysis and had been on dialysis for at least 3 months. Patients were excluded if they had a history of adverse reaction to the study dialyzers, were on home haemodialysis, had a life expectancy <3 months, had severe malnutrition (serum albumin <25 g/l), had malignancy other than non-melanoma skin cancer, had a recent acute coronary syndrome (unstable angina pectoris or acute myocardial infarction in the preceding 3 months) or refusal or inability to provide informed consent for the study. From 140 patients receiving in-centre or satellite haemodialysis, 35 patients were enrolled between October 2002 and July 2003. The research protocol was approved by the Princess Alexandra Hospital Research Ethics Committee.

Study design

The study was a prospective, randomized, crossover group study comparing two groups of haemodialysis patients undergoing SHF and FX-Class high-flux (FXHF) dialysis. The study consisted of two 12 week periods of dialysis with each dialyzer type, both preceded by a 2 week wash-in phase, which used a SHF dialyzer (Figure 1). Patients were randomized to start with FXHF and then change to SHF, or vice versa. Randomization was performed using sealed, sequentially numbered opaque envelopes.

As homocysteine is affected by dietary B-vitamin intake, multivitamin therapy was commenced in all patients for a minimum of 3 weeks prior to commencement of the study, and included folic acid 5 mg daily and vitamin B6 25 mg daily. These supplements were continued for the duration of the study. With the vitamin pre-treatment and then the investigation phase, the total duration of the study was 8 months.

Haemodialysis treatments were carried out using comparable machines. Specifics of treatment, including session duration, blood flow rate, dialysate temperature and flow rate, were prescribed by the attending nephrologist. No changes to the prescription were allowed during the study period with the exceptions of dialysate potassium, calcium and patient’s target weight. Patients were sub-stratified according to their degree of residual renal function, which was measured by a 24 h urine collection. For the purpose of this study, residual renal function was taken as >100 ml urine per day.

Dialyzers used were either standard high-flux, F-series (Fresenius Polysulfone, HF80S, Fresenius Medical Care) or FX-class (advanced Fresenius Polysulfone, FX80, Fresenius Medical Care) and subjected to single use only. The characteristics of the different dialyzers are detailed in Table 1.

The primary endpoint was the pre-dialysis total plasma homocysteine level. The secondary endpoints were

<table>
<thead>
<tr>
<th>Group 1</th>
<th>wash-in* FXHF wash-in SHF</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(2 weeks)</td>
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<tr>
<td></td>
<td>(12 weeks)</td>
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<td>(2 weeks)</td>
</tr>
<tr>
<td></td>
<td>(12 weeks)</td>
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<table>
<thead>
<tr>
<th>Group 2</th>
<th>wash-in SHF wash-in FXHF</th>
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<tbody>
<tr>
<td></td>
<td>(2 weeks)</td>
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<tr>
<td></td>
<td>(12 weeks)</td>
</tr>
<tr>
<td></td>
<td>(2 weeks)</td>
</tr>
<tr>
<td></td>
<td>(12 weeks)</td>
</tr>
</tbody>
</table>

*all wash-in periods used standard high-flux (SHF) dialyser

Fig. 1. Trial protocol.
Table 1. Characteristics of dialyzers by class

<table>
<thead>
<tr>
<th>Dialyzer characteristic</th>
<th>SHF (Fresenius HF80S)</th>
<th>FXHF (Fresenius FX80)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inner lumen (µm)</td>
<td>200</td>
<td>185</td>
</tr>
<tr>
<td>Wall thickness (µm)</td>
<td>40</td>
<td>35</td>
</tr>
<tr>
<td>Surface area (m²)</td>
<td>1.8</td>
<td>1.8</td>
</tr>
<tr>
<td>Fibre packing density (%)</td>
<td>45</td>
<td>60</td>
</tr>
<tr>
<td>Ultrafiltration coefficient (ml/h.mmHg)</td>
<td>55</td>
<td>59</td>
</tr>
<tr>
<td>Sieving coefficients: (QB 300 ml/min, UF 60 ml/min)</td>
<td>Inulin</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>β₂-microglobulin</td>
<td>0.65</td>
</tr>
<tr>
<td></td>
<td>Albumin</td>
<td>0.001</td>
</tr>
<tr>
<td>Clearances: (ml/min at QB 300 ml/min)</td>
<td>Urea</td>
<td>248</td>
</tr>
<tr>
<td></td>
<td>Creatinine</td>
<td>180</td>
</tr>
<tr>
<td></td>
<td>Phosphate</td>
<td>177</td>
</tr>
<tr>
<td></td>
<td>Vitamin B₁₂</td>
<td>135</td>
</tr>
<tr>
<td></td>
<td>Inulin</td>
<td>110</td>
</tr>
</tbody>
</table>

Homocysteine clearance and dialysis adequacy, as measured by equilibrated $K_i/V$ ($eK_i/V$).

Sample collection

Blood collection for homocysteine assays. Plasma fasting total homocysteine concentrations were measured before and after dialysis at the midweek dialysis session. Baseline values were taken during the last week of each wash-in period. During the 3 months of dialysis with each dialysis membrane type blood was collected for homocysteine analysis monthly, immediately prior to the haemodialysis session. Patients were required to fast for 4 hours prior to dialysis and blood collection. A light snack was provided in the first hour of dialysis and patients were then asked to fast for the last 3 hours of dialysis until the post-dialysis blood specimen was collected. Patients remained on either the morning or afternoon dialysis shift for the entire study duration. Five milliliters of blood was collected by Vacutainer® technique into heparinized tubes immediately prior to and also immediately after the haemodialysis session. Plasma samples were then obtained by centrifugation and stored at −70°C until assayed.

Laboratory analyses and calculations

Homocysteine. Total homocysteine was measured using a fluorescence polarization immunoassay (AxSYM; Abbott Laboratory, IL, USA).

MTHFR genotyping. Genomic DNA was isolated from nucleated blood cells by a rapid extraction procedure and the presence of the nucleotide 677 C→T mutation was determined by polymerase chain reaction (PCR) and Hinf I restriction enzyme digestion as described by Frost et al. [15].

Vitamin B₆ and Folate. Vitamin B₁₂ and folate analyses were performed on a Bayer Centaur analyzer, using a competitive binding chemi-luminescent assay.

Vitamin B₆ assay. Vitamin B₆ analysis was performed using the red cell aspartate transaminase activity method (indirect functional assay) on a Cobas Bio analyzer.

Adequacy of dialysis. Adequacy was assessed by a calculated equilibrated $K_i/V$. The single-pool $K_i/V$ was determined by two-point urea modelling on the basis of the intradialytic reduction in blood urea and the intradialytic weight loss [16]. This was corrected to equilibrated $K_i/V$ [17,18].

Homocysteine reduction ratio. This was calculated as (Hcy pre-dialysis – Hcy post-dialysis)/Hcy pre-dialysis.

Statistical methods

Results are expressed as mean±SEM or median and interquartile range for continuous data depending on data distribution, and as frequencies and percentages for categorical data. For comparison of baseline characteristics the Student’s $t$-test or Wilcoxon rank sum test were used depending on data distribution. Differences in proportions were evaluated by the $\chi^2$ or Fisher’s exact test, as appropriate.

The primary end-point analysis (comparison of pre-dialysis Hcy between the two therapies for each patient utilizing the data obtained in week 12 of each study period) and the secondary analyses (comparison of homocysteine reduction ratio and comparison of adequacy using $eK_i/V$) utilized the paired $t$-test. Repeated measures analysis was done using a generalized estimating equations method [19] and incorporated the monthly pre-Hcy levels throughout each study period for each membrane. Adjustments were made for group (FXHF vs SHF as 1st study treatment), baseline Hcy for each patient and MTHFR genotype. Statistical calculations were performed using STATA Version 8 (Stata Corporation, College Station, Texas, USA). An a priori level of $\alpha=0.05$ was taken as significant.

Prospective power calculations demonstrated that a sample size of 35 analysing paired data would have 80% power to determine a difference of 5 µmol/l in pre-dialysis plasma total homocysteine concentration between FXHF and SHF therapies (assuming $\alpha=0.05$ and a standard deviation of 9.9 µmol/l in the two groups). We considered that a decrease in plasma total homocysteine concentration of 5 µmol/l would be a clinically significant result, given the predicted effect on cardiovascular risk of a reduction of this magnitude, which in one meta-analysis was estimated to be similar to a reduction of serum cholesterol concentration of 0.5 mmol/l [1].

Results

Baseline characteristics

Thirty-five patients entered the study, 17 randomized to Group 1 (FXHF followed by SHF) and 18 to Group 2 (SHF followed by FXHF). There were 12 withdrawals (7 in Group 1, 5 in Group 2), thus 10 patients from Group 1 and 13 patients from Group 2 reached the end of the study.
The 35 patients who entered the study were representative of the 140 patients receiving in-centre or satellite haemodialysis in our department at the commencement of the study, as outlined in Table 2. In addition to ensuring patients who entered the study were representative of our dialysis population, we also carried out a detailed comparison of patients who completed the study compared to patients who did not complete the study, also shown in Table 2. The significant differences were that patients not completing the study had significantly lower pre-dialysis baseline Hcy than for patients who remained in the trial (22.81 ± 3.46 vs 27.57 ± 7.02 μmol/l, \(P = 0.017\)), higher vitamin B12 levels (492.78 ± 27.82 vs 408.96 ± 39.04, \(P = 0.041\)), and lower blood flow rates (245 ± 37 vs 266 ± 32, \(P = 0.045\)).

Of the 12 withdrawals from the study the distribution by reason for withdrawal was: serious infection (two); vascular access problems (three); patient withdrawal (two); patient transfer to another facility (two); protocol violation (two) and death (one). The only death during the study was from CVD. No patient withdrew from the trial because of inability to tolerate the FX-class membrane.

**Primary outcome**

There was no difference in pre-HD Hcy with the SHF compared with FXHF dialyzer at the end of 12 weeks, 25.91 ± 5.78 vs 25.00 ± 6.61 μmol/l, 95% CI of the difference −2.77 to 4.59, \(P = 0.31\) (Figure 2 and Table 3). Only patients who completed the trial were included in the primary end-point analysis.

**Secondary outcomes**

There was a non-significant trend toward a decrease in Hcy in both groups, 27.43 ± 7.68 to 25.91 ± 5.78 μmol/l, \(P = 0.23\) for SHF and 26.0 ± 4.58 to 25.0 ± 6.61 μmol/l, \(P = 0.28\) for FXHF (Figure 2 and Table 3). Analysis by repeated measures method after adjusting for group (SHF vs FXHF dialyzer as first treatment),
baseline Hcy and MTHFR genotype, showed a statistically significant fall in pre-dialysis Hcy with FXHF compared with SHF, adjusted \( b = -1.30 \), 95% CI \(-2.41\) to \(-0.19\), \( P = 0.022 \). The interpretation of this result is that treatment with FXHF resulted in a pre-dialysis Hcy that was 1.3\( \mu \)mol/l lower (averaged over all readings during each treatment period) than with SHF (Figure 3).

The homocysteine reduction ratio did not differ between the two different dialysis membranes (SHF and FXHF) for the 12 week periods of dialysis with each membrane, 44.2±10.5% vs 44.4±9.4%; 95% CI of the difference, \(-6.12\) to 6.72; \( P = 0.473 \) (Table 4).

The e\( K_t/V_{urea} \) was significantly higher with the FXHF dialyzer as compared to the SHF dialyzer (1.35±0.18 vs 1.22±0.22, \( P = 0.013 \)) at the end of the 12 week period. (Table 4). There was no significant difference between red cell folate, vitamin B\(_6\), vitamin B\(_{12}\) or albumin levels between SHF and FXHF.

### Discussion

The present study demonstrated no clinically significant benefit of the FX-class dialyzer compared with standard high-flux membranes (F-series) in relation to pre-dialysis plasma homocysteine. The primary outcome analysis, which compared the pre-dialysis Hcy at the end of the 12 week period with the FX-class dialyzer compared with a standard high flux membrane, showed no significant difference. However, as outlined in our secondary analyses using repeated measures analysis, we did detect a significantly lower pre-dialysis Hcy in patients using the FX-class membrane compared to the SHF membrane. The point estimate of the magnitude of the difference was only 1.30\( \mu \)mol/l with 95% CI of 0.19–2.41. We stated a priori that we would regard a difference of 5\( \mu \)mol/l to be clinically significant. Thus, we regard this result as being statistically but not clinically significant.

However, as outlined in our secondary analyses using repeated measures analysis, we did detect a significant difference with patients using the FX-class membrane having a pre-dialysis Hcy 1.30\( \mu \)mol/l lower on average than with standard high flux after adjustment. This small difference is unlikely to be clinically significant, based on the data from published epidemiological studies.

The probability of a type 2 error is very low. Our prospective power calculation showed we needed 35 patients with matched data to have an 80% power to detect a difference of 5\( \mu \)mol/l. However this calculation was based on a standard deviation (SD) of the difference between pre-dialysis Hcy of 9.9\( \mu \)mol/l, whereas in the study the SD of the difference was...
only 3.7 μmol/l: we had a 99% chance of detecting a 5 μmol/l difference and a 90% chance of detecting a 2.6 μmol/l difference. Thus the study was adequately powered to detect a clinically relevant change in plasma Hcy. Although we had 12 withdrawals from the study, a continuous study of 8 months duration in such a population is difficult, due to the inevitable problems of access complications and intercurrent illnesses. Despite this number of withdrawals, we had an adequate number of patients completing the study to provide meaningful results.

The lower SD in the study compared to non-study conditions is likely to reflect uniform prescribing of folic acid and B6 throughout the study and tighter compliance with fasting before blood collection for Hcy assays than for non-trial patients on which the calculation was based. The folic acid–Vitamin B group prescription during the study is the likely explanation for the non-significant trend to a fall in pre-dialysis Hcy seen with either membrane.

Our study does have limitations. In particular, there was a high withdrawal rate of 12/35 (34%). Protocol violations related to fasting, which led to patient withdrawals, and adverse vascular access events were primarily responsible. Because of the high withdrawal rate we compared those who withdrew with those who continued in the study. The patients who did not complete the study had lower Hcy levels compared to those who did (Table 2). We hypothesise that patients who withdrew may have had an increased burden of co-morbidity due to some unmeasured factor, and may have been more predisposed to adverse events or complications necessitating withdrawal. The higher vitamin B12 levels in the patients who withdrew is probably not of clinical significance, as the actual levels were still almost twice the upper limit of the reference range (>210 pmol/l). The lower blood flow rates in patients who withdrew compared to patients who completed the study may perhaps reflect a difference in their cardiovascular tolerance of dialysis, or possibly reflect some subtle difference in the quality of their access. We were not able to elucidate this further, although of note, the types of access and dialysis adequacy assessed by eKt/V were not different in the two groups. In all other respects the patients did not differ. Moreover our study patients were representative of the total cohort of 140 patients from which they were selected, giving further support to the external validity of the study.

Previous studies examining the effect of dialysis membranes on homocysteine status have compared low-flux with high-flux membranes and non-albumin-leaking with albumin-leaking high-flux membranes. Studies comparing low-flux with high-flux therapies have shown variable results [7–9] but the study with the most robust design showed no significant benefit of high-flux over low-flux dialyzers in terms of plasma Hcy pre-dialysis despite greater clearance per dialysis
session [10]. In contrast, studies comparing albumin-leaking and non-leaking membranes showed that albumin-leaking high-flux membranes resulted in a 15% fall at 1 month [12] and up to a 33% decrease in pre-dialysis Hcy at 3 months [11,13]. There has also been an observational study showing Hcy levels to be lower in patients undergoing nocturnal haemodialysis.

To our knowledge this is the only study examining the effect of the FX-class dialyzer on plasma homocysteine. We hypothesized that this new class of polysulfone membrane, designed to eliminate large uraemic substances with high efficiency without excessive leaking of useful proteins such as albumin, might pose an advantage in terms of Hcy status without the disadvantage of excessive albumin losses. The development of this membrane utilised nanotechnology fabrication principles to result in membrane characteristics with minimal resistance to the removal of large molecular weight substances across the membrane, and a membrane claimed to be closer in structure to that of the human glomerulus.

However, our study demonstrated that the new FX-class membrane is not superior to the F-series high-flux dialyzer in terms of homocysteine clearance and did not demonstrate the benefit seen with albumin-leaking dialyzers. The homocysteine reduction ratio in our study was not lower than published studies examining albumin-leaking membranes. In our study the percentage change in Hcy was 44.2 ± 10.5% at the end of 12 weeks with the FX membrane and similar at the percentage change in Hcy was 44.2±10.5% at the end of 12 weeks with the FX-class dialyzer than the F-series dialyzer with no change in any other aspect of the dialysis prescription including hours, blood flow or dialysis access type. The difference is likely to be related to differences in membrane characteristics between the standard and advanced Fresenius polysulfone membranes (Table 1).

Our study lends further weight to the hypothesis that substances thought to affect Hcy metabolism are likely to be highly protein bound or have a molecular weight above that removed by the FX-class dialyzer (i.e. above 15 000 Da), as the ability of the FX-class dialyzer to more effectively clear molecules in the middle molecule range did not result in improvement in homocysteine status whilst albumin-leaking dialyzers do improve Hcy status.

The contemporary debate about whether the optimum dialysis membrane should or should not leak some albumin is an important one. The concern is that albumin-leaking membranes may contribute to hypoalbuminaemia and malnutrition [14]. The potential benefits, though, include not only improved Hcy status but also improvements in anaemia [20]. However, to date we do not have access to data from adequately conducted trials with more appropriate end-points to determine whether albumin-leaking dialyzers will prove more beneficial than the more conventional non-albumin-leaking type.

In conclusion, our study showed no clinically significant benefit of FX-class dialyzers over F-series (standard high-flux) dialyzers in stable vitamin replete haemodialysis patients in relation to achieving a significant correction in pre-dialysis plasma homocysteine concentration. Our data gives further support to the hypothesis that putative solutes affecting Hcy metabolism are likely to be of high molecular weight or be highly protein-bound. However the importance of Hcy status still awaits the results of robust trials exploring relevant outcomes with effective interventions in dialysis-dependent patients.

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Conflict of interest statement. None declared

References


17. Daugirdas JT, Schneditz D. Overestimation of hemodialysis dose depends on dialysis efficiency by regional blood flow but not by conventional two pool urea kinetic analysis. *ASAIO J* 1995; 41: M719–M724


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