Oxidized low-density lipoprotein in plasma is a prognostic marker of subclinical atherosclerosis development in clinically healthy men

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Objective. To investigate the association between plasma oxidized low-density lipoprotein (OxLDL) and the progress of clinically silent atherosclerosis, as measured by ultrasound in the carotid arteries.

Design. Prospective, observational study with more than 3 years of follow-up.

Setting. One-centre study at university hospital.

Material and methods. The subjects (n = 326) were obtained by stratified sampling from a population sample of men who were 58 years old at baseline. Carotid artery intima-media thickness (IMT) was measured bilaterally by high-resolution B-mode ultrasound at baseline and after follow-up. Plasma OxLDL cholesterol concentrations and conventional cardiovascular risk factors were measured at study entry. Automated measurements of IMT were performed. Plaque occurrence and size were assessed (plaque status). Plasma OxLDL at entry was measured by a specific monoclonal antibody, mAb-4E6.

Results. OxLDL at entry, but not LDL cholesterol, was associated with the number and size of plaques at follow-up (P = 0.008), also after adjustment for plaque status at entry (P = 0.033). The plasma OxLDL concentration at entry was associated with change in carotid artery IMT (r = 0.17; P = 0.002) and in a stepwise multiple regression analysis this association remained after adjustment for other cardiovascular risk factors (P = 0.005).

Conclusions. These results provide new information, supporting the concept that circulating OxLDL was associated with the silent phase of atherosclerosis progression in clinically healthy men independently of conventional risk factors.

Keywords: atheroma, atherosclerosis, LDL cholesterol, ultrasonography.

Introduction

Inflammation plays an important role in atherosclerosis development [1]. Recently, research in this field has focused on the role of modified lipoproteins, primarily oxidized low-density lipoprotein (OxLDL). Several lines of evidence support the concept that OxLDL may be a key antigen in atherosclerosis [2]. OxLDL as well as antibodies against epitopes of OxLDL have been found in several studies in both human and rabbit plasma and in atherosclerotic lesions [3–6]. Until recently it has not been possible to measure circulating OxLDL in human plasma. However, Holvoet et al. have, in a number of studies, shown that OxLDL is related to coronary artery disease in heart transplant patients [7, 8], as well as in patients with established coronary artery disease [9, 10]. Furthermore, Toshima et al. recently
showed that OxLDL was higher in subjects with established coronary heart disease when compared with controls [11].

These results were obtained in selected patients with either overt clinical disease or with angiography-proven atherosclerosis. With such approaches it is not possible to study mechanisms related to the very long clinically silent phase of atherosclerosis when the intravascular lumen often is normal. The development of the B-mode ultrasound technique has made it possible to noninvasively study early artery wall changes in the atherosclerotic process. Intima-media thickness (IMT) of the carotid artery has been used as a noninvasive indicator for the atherosclerotic process in the coronary arteries and as a predictor for future cardiovascular disease [12, 13]. IMT of the carotid bulb and plaque occurrence and size in the carotid artery are associated with coronary atherosclerosis, as measured by coronary angiography [14].

Using this technique we recently showed that OxLDL is also related to subclinical atherosclerosis in a cross-sectional, population-based study consisting of clinically healthy, middle-aged men [15]. A limitation with cross-sectional studies is, however, that no conclusions can be drawn about causality. As yet no population-based, prospective study has examined the role of circulating levels of OxLDL in relation to progress of subclinical atherosclerotic disease. Hence, the aim of the present study was to investigate the relationship between 3-year progress of clinically silent atherosclerosis, as measured by ultrasound in the carotid artery, and baseline values of OxLDL. These observations were made in clinically healthy 58-year-old men with varying degrees of obesity and fasting blood glucose obtained from the general population.

Study population and methods

Study group

The design was a longitudinal study based on a randomly selected population sample of clinically healthy men (n = 818). As previously described in detail a stratified sampling procedure was used to include men with different degrees of obesity and insulin sensitivity (n = 391) [16]. Briefly, the screened subjects were divided into quintiles of an estimate of insulin sensitivity. This estimate was an algorithm that was based on body mass index and fasting blood glucose, showing high correlations with insulin sensitivity as measured by the euglycemic, hyperinsulinemic clamp method [16]. All subjects in the lowest and highest quintiles of the estimate were included, whereas every fifth subject in quintiles 2–4 was randomly selected.

The inclusion criteria at baseline were male sex, age 58 years and Swedish ancestry. Exclusion criteria were cardiovascular disease (myocardial infarction, angina pectoris, stroke, intermittent claudication and aortic disease), clinical diabetes mellitus or other established disease, treatment with cardiovascular drugs (i.e. anti-diabetic, lipid-lowering, antihypertensive, heart failure drugs or drugs due to angina pectoris), which might disturb the measurements performed in the study, or unwillingness to participate. No subjects with clinically overt diabetes were included.

This study reports on the 3-year follow-up examination that was performed in order to examine the change in carotid artery IMT and plaque status and to relate these changes to the baseline characteristics. The mean follow-up time was 3.2 ± 0.2 years. We were able to re-examine 342 of the originally group of 391 men and 326 of those men had requested data of OxLDL at entry, and either plaque occurrence or intima-media measurements available. The reasons for being excluded from the present analysis were death (n = 4), incomplete data (n = 12), having moved or refusal to participate (n = 45).

The subjects received both written and oral information before they gave their consent to participate. The study was approved by the Ethics Committee at Sahlgrenska University Hospital.

Ultrasonography

Intima-media thickness. Examination was performed with an ultrasound scanner (Acuson 128, Acuson Corporation, Mountain View, CA, USA) with a 7-Mhz linear transducer aperture of 38 mm. The electrocardiographic signal (lead II) was simultaneously recorded to synchronize the image capture of the top of the R-wave to minimize variability during the cardiac cycle. A 10-mm segment of both the left and right carotid arteries was scanned at the level of the bifurcation and images for IMT measurements were recorded from the far wall in the
common carotid artery and the carotid artery bulb. The software program gives the average as well as the maximal thickness of the IMT on the left and right side for the common carotid artery and the carotid artery bulb, respectively. Hence, four variables are defined, namely IMT mean and maximum in the common carotid artery and carotid artery bulb. IMT was defined as the distance from the leading edge of the lumen-intima interface to the leading edge of the media-adventitia interface of the far wall. At the position of the thickest part of the wall (visually judged), a frozen longitudinal image was captured and recorded on videotape. A short sequence of real-time images was also recorded on videotape to assist in the interpretation of the frozen images.

The images were measured in an automated analysing system [17], based on automatic detection of the echo structures in the ultrasound image but with the option to make manual corrections by the operator. A composite measure of IMT in the carotid artery was calculated as the mean of the maximum IMT in the common carotid and the carotid artery bulb on the left and right side (composite IMT). The interobserver variability for measurement of IMT in the common carotid artery, and the carotid artery bulb was 5.3 and 6.0%, respectively [18], and it was 7.6% for the composite measure. The laboratory technician was blinded to the previous examination.

Assessment of plaque occurrence. The carotid arteries were scanned both longitudinally and transversely to assess the occurrence of plaques [19]. A plaque was defined as a distinct area with an IMT more than 50% thicker when compared with neighbouring sites (visually judged). A semi-quantitative subjective scale was used to grade the size of plaques into grade 1: one or more small plaques (less than approximately 10 mm². The plaque area was automatically calculated by the ultrasound machine after manual tracing of the plaque boundaries.); grade 2: moderate to large plaques (the differentiation between grades 1 and 2 was made subjectively in most cases, and quantitative measurements were made in the computerized system [20] only when the correct classification was not obvious to the observer); grade 3: plaques giving flow disturbances [17].

In the present study two subjects at baseline and three subjects at follow-up had plaques of grade 3 in the carotid artery. Therefore, plaques of grade 2 and 3 were merged into one group of moderate-to-large plaques. This analysis included plaques in the near wall as well as the far wall of the vessel. Analyses of plaques were performed in both the right and left carotid arteries. The largest plaque in either artery was used in the present analysis. However, subjects with plaque grade 0 or 1 on either side at baseline or follow-up, but with the other side missing at entry or follow-up, respectively, were excluded from the analyses. In a re-reading reproducibility study (n = 45) of plaque size there were high correlation coefficients for both the right and left carotid arteries ($r_s = 0.96$ and $r_s = 0.96$ respectively).

Measurements

All measurements were performed in the morning. Information on general health and smoking habits were obtained by a self-administered questionnaire. Current smoking was defined as everyday use of at least one cigarette per day. Ex-smoking was defined as no use during the last 3 months. The total number of years of smoking was multiplied by the number of cigarettes smoked daily. The product was called ‘cigarette-years’.

Blood pressure was measured twice when the subject had been resting in the supine position for 5 min with appropriate cuff size in relation to arm size. The diastolic blood pressure was determined as Korotkoff phase V. A 12-lead standard electrocardiogram (ECG) was recorded. Heart rate was recorded from the ECG.

Biochemical analysis

Blood samples for serum cholesterol, serum triglycerides and lipoprotein fractions were drawn after a fasting period of 10–12 h; serum was separated and frozen within 4 h at −70 °C. Cholesterol and triglyceride levels were determined by fully enzymatic techniques [21, 22]. LDL cholesterol was calculated as described by Friedewald et al. [23]. OxLDL concentration in plasma, that had been stored at −70 °C, was measured by a sandwich ELISA (Mercodia, Uppsala, Sweden) utilizing the same specific murine monoclonal antibody, mAb-4E6, as in the assay described by Holvoet et al. [9]. Using this antibody (mAb-4E6) it is possible to measure very small amounts OxLDL containing a conformational
epitope in the apoB-100 moiety of LDL that is generated as a consequence of substitution of lysine residues of apoB-100 with aldehydes [7]. The specificity for the antibody mAb-4E6 has been assessed, showing that 50% inhibition of binding of mAb-4E6 to immobilized OxLDL was obtained with 0.025 mg dL \(^{-1}\) Cu\(^{2+}\) OxLDL and with 25 mg dL \(^{-1}\) native LDL [24].

In the presently used sandwich ELISA, the plates were coated with the capture antibody (mAb-4E6), and the secondary antibody specifically detects apoB. In a set of 20 EDTA plasma samples collected from 20 different individual blood donors, no unspecific binding of native LDL to the solid phase in the Mercodia Qxidized LDL ELISA system was detected (data not shown). The precision has been satisfactory [15]. In order to avoid systematic differences in the present study two internal controls were repeatedly included on all plates \((n = 10)\). Mean values and standard deviations for the two controls were 38.4 \(\pm\) 2.5 U L \(^{-1}\) (range 35.3–43.8) and 83.1 \(\pm\) 4.3 U L \(^{-1}\) (range 77.8–89.6). All analyses were performed at the Wallenberg Laboratory.

Comparison of baseline characteristics between the men in whom it was possible to obtain follow-up data \((n = 326)\) and those in whom it was not showed no statistically significant difference in OxLDL \((85.8 \pm 24.0\) U L \(^{-1}\) vs. 88.3 \(\pm\) 25.5 U L \(^{-1}\); \(n = 60; P = 0.46)\) or composite IMT \((1.17 \pm 0.24\) mm vs. 1.25 \(\pm\) 0.33 mm; \(n = 56, P = 0.08)\). The men who were not re-examined tended to have more small and moderate-to-large atherosclerotic plaques \((26\% vs. 18\% and 35\% vs. 24\%, P = 0.051)\) than those who participated.

We succeeded in obtaining information on the morbidity in all of the re-examined subjects, with the exception of one man. The cardiovascular morbidity in the re-examined group \((n = 326)\), and the not re-examined group \((n = 65)\), at the time of follow-up was 5.2 and 9.2\% respectively (NS).

Statistics
All statistics were analysed by using SPSS for Windows 10.0 (Chicago, IL, USA). The 95\% confidence intervals were calculated for annual change in IMT. Simple Spearman’s rank correlation coefficients were used to calculate univariate associations. Skewed variables were logarithm-transformed before statistical testing. Multiple regression analysis was used to explore the associations between the change in carotid artery IMT and characteristics at study entry. The correlation between patient characteristics at baseline and plaque status at the follow-up examination with adjustment for plaque status at baseline was calculated by Mantel’s test. The correlations between the mentioned variables and follow-up time were tested by Pitman’s test. \(P < 0.05\) (two-tailed) was regarded as statistically significant.

Results
The baseline characteristics of the subjects included in the present study were representative of the underlying clinically healthy population sample presented in Table 1.

**IMT and plaque occurrence at entry and follow-up**

The results of the ultrasound examinations are illustrated in Table 1. During follow-up there was a statistically significant increase in the composite IMT measure \([47 (95\% CI 29–66)\) \(\mu\)m year \(^{-1}\), \(P < 0.001)\). More and larger plaques tended to occur during follow-up \((P = 0.051)\). Thus, in comparison with baseline, the follow-up examination showed that there were less subjects with no plaque \((179 vs. 173)\) as well as small plaques \((54 vs. 50)\), and more subjects had moderate-to-large atherosclerotic plaques \((74 vs. 87)\).

**Change in IMT in relation to OxLDL and other variables at entry**

Figure 1 shows that the tertiles of OxLDL at entry were associated with an increase in composite IMT during follow-up.

As presented in Table 2, the change in composite IMT correlated with OxLDL, LDL cholesterol and HDL cholesterol at entry. In a stepwise multiple regression model with the change in composite IMT as dependent variable and OxLDL, LDL and HDL cholesterol, smoking and systolic blood pressure as covariates, only OxLDL was significantly associated with the annual change in composite IMT (partial correlation coefficient 0.16, \(P = 0.005)\). In a covariate analysis with the change in composite IMT as dependent variable, the partial correlation coefficients were 0.09 \((P = 0.13)\) and 0.04 \((P = 0.44)\).
for OxLDL and LDL cholesterol respectively. OxLDL correlated with total cholesterol \( (r = 0.66) \) and LDL cholesterol \( (r = 0.65, \text{ both } P\text{-values} < 0.001) \). In order to separate the effects of OxLDL and LDL cholesterol on change in IMT a 3.4-time larger study is needed \( (z = 0.05, \beta = 0.20) \).

### Table 1
Characteristics of the study subjects at entry \( (n = 326) \). Mean and SD are given if nothing else is indicated.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Population sample ( (n = 818) )</th>
<th>Present study ( (n = 326) )</th>
</tr>
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<tbody>
<tr>
<td>Body mass index (kg m(^{-2}))</td>
<td>26.2 ± 3.3</td>
<td>26.3 ± 4.3</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94 ± 0.06</td>
<td>0.94 ± 0.06</td>
</tr>
<tr>
<td>Present smoker [n (%)]</td>
<td>188 (23)</td>
<td>66 (20)</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>136 ± 19</td>
<td>134 ± 18</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>82 ± 10</td>
<td>81 ± 10</td>
</tr>
<tr>
<td>Blood glucose (mmol L(^{-1}))</td>
<td>4.9 ± 1.0</td>
<td>4.9 ± 1.1</td>
</tr>
<tr>
<td>Serum insulin (mU L(^{-1}))</td>
<td>9.4 ± 5.2</td>
<td>9.5 ± 5.0</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total (mmol L(^{-1}))</td>
<td>6.04 ± 1.09</td>
<td>6.01 ± 1.10</td>
</tr>
<tr>
<td>HDL (mmol L(^{-1}))</td>
<td>1.27 ± 0.35</td>
<td>1.28 ± 0.36</td>
</tr>
<tr>
<td>LDL (mmol L(^{-1}))</td>
<td>4.07 ± 0.97</td>
<td>4.08 ± 0.97</td>
</tr>
<tr>
<td>Serum triglycerides (mmol L(^{-1}))</td>
<td>1.35 (0.41–11.15)</td>
<td>1.28 (0.41–9.89)</td>
</tr>
<tr>
<td>(median:min–max value)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxidized LDL (U L(^{-1}))</td>
<td>–</td>
<td>85.8 ± 24.0</td>
</tr>
<tr>
<td>Composite IMT (mm)</td>
<td>–</td>
<td>1.17 ± 0.24</td>
</tr>
<tr>
<td>Atherosclerotic plaques in the carotid artery</td>
<td>–</td>
<td>74 (24)</td>
</tr>
<tr>
<td>No plaques [n (%)]</td>
<td>–</td>
<td>179 (58)</td>
</tr>
<tr>
<td>Small plaques [n (%)]</td>
<td>–</td>
<td>54 (18)</td>
</tr>
<tr>
<td>Moderate to large plaques [n (%)]</td>
<td>–</td>
<td></td>
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</table>

**Fig. 1** Change in the carotid artery IMT during 3 years of follow-up by tertiles of plasma OxLDL concentration at the baseline examination \( (n = 313) \). Mean ± SEM.

### Table 2
Correlations between entry characteristics and change in composite carotid artery during 3 years of follow-up \( (n = 326) \).

<table>
<thead>
<tr>
<th>Entry characteristic</th>
<th>Change in composite IMT ( (n = 326) ) ( (r) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>0.07</td>
</tr>
<tr>
<td>Serum cholesterol</td>
<td></td>
</tr>
<tr>
<td>Total (mmol L(^{-1}))</td>
<td>0.11</td>
</tr>
<tr>
<td>LDL (mmol L(^{-1}))</td>
<td>0.13*</td>
</tr>
<tr>
<td>HDL (mmol L(^{-1}))</td>
<td>-0.12*</td>
</tr>
<tr>
<td>Serum triglycerides (mmol L(^{-1}))</td>
<td>0.10</td>
</tr>
<tr>
<td>Cigarette-years</td>
<td>-0.05</td>
</tr>
<tr>
<td>OxLDL (U L(^{-1}))</td>
<td>0.17**</td>
</tr>
</tbody>
</table>

\*\( P < 0.05 \), **\( P < 0.01 \).

**Fig. 2** Upper panel: relationship between plaque status after 3 years of follow-up and plasma OxLDL concentration at entry \( (n = 310) \). Mean ± SEM. Lower panel: relationship between plaque status after 3 years of follow-up and serum LDL concentration at entry \( (n = 307) \). Mean ± SEM.

**Plaque status in relation to OxLDL and LDL at entry**

Plasma OxLDL, but not serum LDL, concentration at entry was associated with plaque status at the follow-up examination (Fig. 2). Hence, occurrence of plaque and plaque size at the follow-up examination...
was associated with higher OxLDL concentrations at baseline. Mantel’s test showed that OxLDL concentration \( (P = 0.033) \) and systolic blood pressure \( (P = 0.004) \) at study entry were associated with plaque status at the follow-up examination independent of initial plaque status. Neither serum concentrations of LDL cholesterol, HDL cholesterol or triglycerides at baseline showed such associations with plaque status (all \( P > 0.50 \)). OxLDL and systolic blood pressure were not associated.

**Discussion**

The results from this prospective 3-year study show that circulating OxLDL was associated with the progression of ultrasound-assessed atherosclerosis, independent of conventional risk factors in a cohort of clinically healthy 58-year-old men with varying degrees of obesity and fasting blood glucose obtained from the general population by a stratified sampling procedure. These findings extend our knowledge of the importance of OxLDL in human atherosclerosis as prospective data have so far been limited to transplant-associated coronary disease [8].

The ultrasound technique is well established in practice since many years and our research group has developed a computerized reading system that is associated with a high reproducibility for measuring IMT [17]. A plaque-scoring method has also been developed and used in several studies [15, 20].

The plasma OxLDL concentration at baseline was associated with the occurrence and size of atherosclerotic plaques in the carotid arteries after 3 years, independently of plaque status at entry and the other measured serum lipids. In addition, the progression of carotid artery IMT during follow-up was associated with baseline OxLDL in a stepwise multiple regression, including all conventional risk factors. Due to co-variability a more than three times larger study than the present one is needed in order to clarify the separate effects of OxLDL and LDL cholesterol on IMT.

As shown in a previous study using another method with the mAb-4E6 antibody it was possible to measure very small amounts of OxLDL containing a conformational epitope in the apoB-B-100 moiety of LDL that is generated as a consequence of substitution of lysine residues of apoB-100 with aldehydes [7]. To some extent the mAb-4E6 also detects circulating MDA-LDL. Our study was not designed to examine the occurrence and importance of other epitopes of OxLDL. However, OxLDL is a very complicated particle and the measurement of its nature at a single epitope has the inherent problem of not recognizing this heterogeneity. Hence, it might well be that other epitopes on the LDL particle, such as oxidized phospholipids, carry important information with regard to subclinical atherosclerosis development. A previous study using an antibody recognizing oxidized phosphatidylcholine, which is another epitope to that used in the present study, also reported an association between OxLDL and coronary heart disease [11]. In a study using a monoclonal antibody binding to oxidized phospholipids acute coronary syndrome was associated with an increase in circulating OxLDL-specific markers [25]. In patients operated for carotid artery stenosis, pravastatin treatment was associated with a lower concentration of OxLDL in the excised plaque tissue than those not receiving such therapy [26].

OxLDL, as measured by using mAb-4E6, accumulated in coronary atherosclerotic lesions in hypercholesterolemic rabbits [27], hypercholesterolemic miniature pigs [28] and patients with coronary artery disease [9]. A number of cross-sectional studies have shown that OxLDL, measured by the presently used antibody (mAb-4E6), is associated with coronary artery disease [7, 11, 15]. The measurement of OxLDL has also been shown to improve cardiovascular risk prediction [10]. We have previously published results from a cross-sectional study of this cohort of 58-year-old men demonstrating that OxLDL was associated with IMT and plaque occurrence in the carotid and femoral arteries [15]. In addition, OxLDL was associated with plasma concentrations of several molecules, which participate in the atherosclerotic process: adhesion molecules, proinflammatory cytokines and C-reactive protein [15]. Experimental studies in rabbits [27] and miniature pigs [28] have also indicated that OxLDL, as measured by the mAb-4E6 technique, is associated with the growth of the atherosclerotic plaque. Atherosclerosis is a heterogeneous disease process including both a slow progress and sudden plaque instability, leading to acute coronary syndromes. Available data indicate that mAb-4E6 is more related to the atherosclerotic process and less to plaque instability [9].
The limitation of the present study is that only clinically healthy 58-year-old Caucasian men with varying degrees of obesity and insulin resistance were studied. The rationale was to study men with insulin resistance, a key factor in the metabolic syndrome, and to select subjects in an age span when atherosclerotic disease is beginning to increase in prevalence, and to reduce a number of potentially confounding factors, e.g. different ages, sex, concomitant disease and accompanying medication. This category of men fulfilling criteria for the metabolic syndrome have high plasma OxLDL concentrations compared with controls [29]. Sixty-five men were not re-examined, and this is also a potential limitation. However, the characteristics of the men included in the present study were very representative of the underlying clinically healthy population sample and there was no statistically significant difference at baseline between those who were and were not re-examined with regard to OxLDL, composite IMT or cardiovascular morbidity.

One important issue not addressed in the present study is whether OxLDL has similar or even better properties to predict the development of atherosclerotic disease in the general population, and in high-risk patients with conventional cardiovascular risk factors, or already known atherosclerotic organ damages. Such studies have to be much larger than the present study due to many confounders, e.g. concomitant treatment, different sexes or varying ages. The underlying concept of the present study is to use an approach that allows preliminary testing of a hypothesis on a small scale. Hence, further studies are needed.

In summary, the present study shows that in a cohort of clinically healthy 58-year-old men plasma concentrations of OxLDL were associated with the progress of atherosclerosis in the carotid arteries during 3 years of follow-up, independently of LDL cholesterol and other conventional risk factors.

Conflict of interest statement
No conflicts of interest exist for the authors.

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References


27 Holvoet P, Collen D. Beta-VLDL hypercholesterolemia relative to LDL hypercholesterolemia is associated with higher levels of oxidized lipoproteins and a more rapid progression of coronary atherosclerosis in rabbits. Arterioscler Thromb Vasc Biol 1997; 17: 2376–82.


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