Elevated Cystatin C Concentration and Progression to Pre-Diabetes

The Western New York Study

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OBJECTIVE — We conducted a nested case-control investigation to examine whether elevated baseline concentrations of cystatin C predicted progression from normoglycemia to pre-diabetes over 6 years of follow-up from the Western New York Health Study.

RESEARCH DESIGN AND METHODS — In 2002–2004, 1,455 participants from the Western New York Health Study, who were free of type 2 diabetes and known cardiovascular disease at baseline (1996–2001), were reexamined. An incident case of pre-diabetes was defined as an individual with fasting glucose <100 mg/dl at the baseline examination and ≥100 and ≤125 mg/dl at the follow-up examination, thereby eliminating individuals with prevalent pre-diabetics. All case patients (n = 91) were matched 1:3 to control participants based on sex, race/ethnicity, and year of study enrollment. All control subjects had fasting glucose levels <100 mg/dl at both baseline and follow-up examinations. Cystatin C concentrations and the urinary albumin-to-creatinine ratio were measured from frozen (−196°C) baseline blood and urine samples. Serum creatinine concentrations were available from the baseline examination only.

RESULTS — Multivariate conditional logistic regression analyses adjusted for age, baseline glucose level, homeostasis model assessment of insulin resistance, BMI, hypertension, estimated glomerular filtration rate, cigarette smoking, and alcohol use revealed a significantly increased risk of progression to pre-diabetes among those with elevated baseline concentrations of cystatin C (odds ratio 3.28 [95% CI 1.43–7.54]) (upper quintile versus the remainder). Results of secondary analyses that considered high-sensitivity C-reactive protein, interleukin-6, E-selectin, or soluble intercellular adhesion molecule-1 did not alter these results.

CONCLUSIONS — These results suggest that cystatin C was associated with a threefold excess risk of progression to pre-diabetes in this population.

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Recent evidence from randomized clinical trials (1,2) among people with pre-diabetes have provided convincing evidence that early intervention can significantly delay or prevent the progression to type 2 diabetes. The identification of those with pre-diabetes is assuming greater importance (3), especially in light of the fact that ~37% of adults aged 40–74 years in the U.S. have pre-diabetes defined as impaired fasting glucose (4). Microalbuminuria occurs frequently in nondiabetic subjects and places them at increased risk for cardiovascular disease (CVD) (5–7). The mechanisms behind this observation are poorly understood, however. Albuminuria may reflect underlying vascular damage (8), hypertension (9,10), endothelial dysfunction (11,12), and/or low-grade inflammation (13).

A large percentage of individuals with type 2 diabetes pass through a period of pre-diabetes (14) and may experience early renal dysfunction, e.g., a glomerular filtration rate (GFR) >60 ml/min per 1.73 m^2. Currently used estimating equations are poor at identifying early renal impairment and better indexes are of great interest (15,16). Recently, several studies have suggested that cystatin C levels may be a more sensitive marker of early renal impairment than either albuminuria or serum creatinine concentration (17–20). Cystatin C is a novel measure of kidney function that seems to overcome the limitations of serum creatinine concentration. Cystatin C is a cysteine protease inhibitor that is produced by virtually all nucleated cells and released into the bloodstream. It is entirely filtered by the kidney glomerulus and metabolized by the proximal tubule (21). Many studies have shown that cystatin C may be a more sensitive indicator of mild renal impairment and may better estimate the GFR than serum creatinine (22). Moreover, concentrations of cystatin C are not affected by sex, age, or muscle mass (23). Therefore, a better understanding of a putative role for cystatin C in the etiology of pre-diabetes could shed light on the renal/heart disease connection (24). Given the reported superiority of cystatin C over conventional measures of renal function, we hypothesized that cystatin C would predict progression to pre-diabetes independent of serum creatinine or estimated GFR (eGFR). We also investigated the role of intervening mechanisms including hypertension, insulin resistance, endothelial dysfunction, and inflammation.

RESEARCH DESIGN AND METHODS

— The study design and methodology of this community-based investigation have been published previ-

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Abbreviations: ACR, albumin-to-creatinine ratio; CVD, cardiovascular disease; DPP, Diabetes Prevention Program; eGFR, estimated glomerular filtration rate; GFR, glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; IL-6, interleukin-6; sE-selectin, soluble E-selectin; HOMA-IR, homeostasis model assessment of insulin resistance; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; sICAM-1, soluble intercellular adhesion molecule-1.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

ously (25,26). Briefly, we enrolled healthy residents from Erie and Niagara Counties, New York, into the Western New York Study. These participants had no known CVD or cancer and were examined between 1996 and 2001. The initial cohort of participants was randomly selected from drivers' license lists for those aged <65 and from the Health Care Finance Administration rolls for those aged 65–79. In 2001–2004, we conducted the first follow-up of the group without known CVD. Eligible participants for the follow-up study were men and women aged 39–79 years selected from the baseline examination without known clinical CVD (self-reported myocardial infarction, angina, or revascularization surgery) or type 2 diabetes (measured fasting plasma glucose >125 mg/dl or self-report and taking medication) who were capable of completing the current study protocol (n = 2,652). Exclusion criteria also included self-report of a medical condition that would prohibit participation (e.g., all cancers except skin cancer, type 1 diabetes, or physical or mental impairment), permanent change in residence out of state, deceased, or an inability to contact and determine eligibility. This left 2,139 individuals eligible for this examination of whom 1,455 completed the full clinic protocol (68.0% response rate). Compared with those who refused, the participants were less likely to smoke and had more formal education. There were no significant differences in fasting glucose concentrations or BMI or sex ratio (data not presented). The mean ± SD follow-up time was 5.9 ± 0.8 years. The protocol was approved by the University at Buffalo Health Science Institutional Review Board, and all participants provided written informed consent before participation.

Study protocol

At both the baseline and 6-year follow-up examinations, all participants received a clinical examination that included resting blood pressure, measures of height, weight, and sagittal abdominal girth according to standardized protocols (27). Hypertension was defined as a systolic blood pressure ≥140 mmHg or a diastolic blood pressure ≥90 mmHg or use of antihypertensive medications regardless of blood pressure level. Study subjects also provided a fasting (at least 10 h overnight) blood sample and were asked to refrain for 24 h from smoking or vigorous physical activity. Several standardized questionnaires were administered including cigarette use (never or ever) and lifetime pack-years of smoking, physical activity (Stanford 7-Day Recall), alcohol use, general health and well-being, personal and family health history, and socioeconomic status. The participants were asked to bring all prescription and over-the-counter medications with them to the clinic to identify hypertension, type 1 or type 2 diabetes, and other comorbid conditions. A positive family history of type 2 diabetes was defined as a positive report in a first-degree relative. An incident case of pre-diabetes was defined as an individual with fasting glucose <100 mg/dl at the baseline examination and ≥100 and ≤125 mg/dl at the follow-up examination. All case patients (n = 91) were matched 1:3 to control participants based on sex, race/ethnicity, and year of study enrollment. All control subjects had fasting glucose levels <100 mg/dl at both baseline and follow-up examinations. Thus, all individuals with prevalent pre-diabetes at the baseline examination were excluded in this report. No one reported history of gestational diabetes in this report.

Laboratory methods

Fasting glucose concentrations were determined by the glucose oxidase method (Beckman, Fullerton, CA). The interassay coefficient of determination was <5%. After identification of those who did (case patients) or did not (control subjects) develop pre-diabetes, the baseline aliquots of serum or plasma were retrieved and sent by overnight courier for analysis. Cystatin C was measured using the BNII nephelometer (Dade Behring, Deerfield, IL) using a particle-enhanced immunonephelometric assay (N Latex Cystatin C test kit). This assay range was 0.195–7.330 mg/dl. The intra-assay coefficient of variance (CV) range was 2.0–2.8% and the interassay range was 2.3–3.1%. Interleukin-6 (IL-6) was measured by an ultrasensitive ELISA (R&D Systems, Minneapolis, MN). Using this method, we have determined a routine CV in the laboratory of 6.3%. Soluble E-selectin (sE-selectin) was measured using a high-sensitivity quantitative sandwich enzyme (Parameter Human sE-Selectin Immunoassay; R&D Systems). Intra-assay and inter-assay CVs ranged from 4.7 to 5.0 and 5.7 to 8.8%, respectively. Human soluble intercellular adhesion molecule-1 (sICAM-1) was measured by an ELISA (Parameter Human sICAM-1 Immunoassay; R&D Systems). The laboratory CV was 5.0%. High-sensitivity C-reactive protein (hs-CRP) was measured using the BNII nephelometer from Dade Behring using a particle-enhanced immunonephelometric assay. Intra-assay CVs ranged from 2.3 to 4.4%, and inter-assay CVs ranged from 2.1 to 5.7%. Fasting insulin was assayed from a kit provided by Linco that has minimal cross-reactivity with human proinsulin. The assay has a lower detection limit of 2 μU/ml with an interassay CV of 3.6–8.4% and an intra-assay CV of 2.2–4.4%. The homeostasis model of insulin resistance (HOMA-IR) was calculated as fasting glucose × (fasting insulin/22.5) (28). A frozen spot urine sample was also retrieved and used for the assessment of the albumin-to-creatinine ratio (ACR). Collections were done in the morning of the baseline visit and stored on liquid nitrogen. Albumin was assayed with a nephelometric immunoassay using a monospecific antiserum to human albumin. Creatinine concentration was determined using the modified Jaffé method. The urinary ACR (milligrams of albumin per gram of creatinine) was calculated and used as a surrogate of albumin excretion rate (29). Intraclass correlation coefficients among duplicate samples were >0.95. Of the values, >94% were <30 mg/g. eGFR was determined using the four-variable formula from the Modification of Dietary Restriction in Diabetes Study (30,31). This formula is eGFR = $186 \times (\text{serum creatinine concentration}^{-1.154}) \times (\text{age}^{-0.203}) \times 1.21$ (if black) or × 0.742 (if female). We did not measure these explanatory variables at the follow-up examination.

Statistical procedures

For this report, data were compared between case patients and control subjects with χ^2 tests or unpaired t tests as appropriate. Multivariate conditional logistic regression models (conditioned on the matching variables) were used to estimate the association between elevated cystatin C and pre-diabetic status after adjustment for age (model 1) and also after adjustment for several explanatory variables (model 2). We a priori compared the top quintile of cystatin C to the remaining 80% determined by the distribution of cystatin C among the control subjects. The top quintile range was 1.02–1.70 mg/dl. Of the case patient group, >50% arose from the top quintile. Tests for linear trend were examined by including cystatin C as a continuous variable. For ACR, we chose a cut point of 20 and for serum

Table 1—*Selected baseline characteristics among prediabetic case patients and matched control subjects*

	Case patients	Control subjects	*P* value
n	91	273	
Age (years)	57.9 ± 11.0	54.2 ± 10.8	0.005
BMI (kg/m$^{2)}$	28.1 ± 5.2	26.9 ± 4.6	0.040
Waist (cm)	91.9 ± 13.4	87.2 ± 12.5	0.003
Abdominal height (cm)	21.2 ± 3.2	20.1 ± 3.2	0.005
Lifetime total pack-years (cigarettes)	14.4 ± 22.6	9.1 ± 15.8	0.037
Physical activity (METs in past 7 days)	259.1 ± 46.3	262.4 ± 49.8	0.574
Glucose (mg/dl)	94.2 ± 4.3	90.4 ± 5.4	<0.001
HOMA	3.4 ± 1.7	2.9 ± 1.5	0.011
ACR (mg/g)	12.0 ± 37.4	7.3 ± 10.4	0.256
Cystatin C (mg/l)	1.0 ± 0.2	0.9 ± 0.1	<0.001
eGFR (ml/min per 1.73 m^2)*	83.0 ± 17.7	85.3 ± 17.0	0.275
Serum creatinine (g/l)	0.8 ± 0.1	0.8 ± 0.1	0.579
Male sex (%)	42.9	42.9	—
White (%)	95.6	95.6	—
Education >12 years (%)	59.3	71.1	0.038
Family history of diabetes (%)	42.9	26.7	0.005
Hypertension (%)	38.6	20.8	0.001
Family history of hypertension (%)	39.3	35.7	0.549
Smoking (%)			0.017
Never	37.4	51.8	
Ever	62.6	48.2	
Drinking (%)			0.028
Abstainer	7.7	9.7	
Non-current	30.8	20.4	
Non-daily	50.5	64.7	
Daily	11.0	5.2	

Data are means ± SD or *n* (%). *eGFR = 186 × (serum creatinine$^{-1.154}$) × (age$^{-0.203}$) × 1.21 (if black) or × 0.742 (if female). †Matching variable.

creatinine and eGFR, we split the distribution at the median value of the control subjects (0.8 mg/l and 83.6 ml/min per 1.73 m^2). There were too few people to examine meaningfully the effect of higher clinical cut points. Likelihood ratio tests were conducted to test for statistical interactions by comparing the log likelihood between the two nested models, one with only the main effects and the other with both the main effects and the interaction terms in the model. No significant effect modification was noted. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant. Only subjects with complete data were included in the analyses. Analyses were carried out using SPSS for Windows (version 14.0; SPSS, Chicago, IL).

RESULTS — Table 1 presents selected characteristics at baseline for case patients and control subjects. There were 91 case patients with pre-diabetes who were matched with 273 control participants. Those who developed pre-diabetes were significantly older, more obese, and heavier lifetime smokers than the control subjects ($P < 0.05$ for each). Case patients were also significantly more insulin resistant at baseline and displayed higher mean concentrations of cystatin C. No significant differences were observed for serum creatinine or eGFR. Fasting baseline concentrations of plasma glucose were higher among the case patients than among the control subjects (94.2 vs. 90.4 mg/dl, $P < 0.001$). Case patients had significantly less formal education and a higher prevalence of a family history of diabetes and were more likely to be hypertensive at the baseline examination. Compared with the control subjects, case patients were more likely to have been smokers and consumers of alcohol ($P < 0.05$).

Table 2 presents the results of conditional logistic regression also adjusted for age. As shown, a positive family history of type 2 diabetes, hypertension, cigarette smoking, and HOMA-IR were each related to pre-diabetic status. BMI ≥27 kg/m^2 was associated with a 62% excess risk, although the 95% CI included unity. Neither serum creatinine nor ACR was found to predict pre-diabetic status. Consideration of continuous forms for these variables also provided no evidence of association with pre-diabetic status.

The results of the multiple conditional logistic regression analyses for cystatin C and the odds of developing pre-diabetes are displayed in Table 3. Models were conditioned on the matching variables of sex, race, and year of study enrollment. Model 1 was further adjusted for age, and model 2 was fully adjusted for age and several of the explanatory variables shown in Table 2 including eGFR. Comparing the highest quintile of cystatin C with the lowest 80% revealed an age-adjusted odds ratio (OR) of 5.08 (95% CI 2.69–9.58). After consideration of the putative risk factors for pre-diabetes in model 2, the OR was attenuated to 3.28 (1.43–7.54) but remained highly statistically significant ($P \leq 0.01$). A test for linear trend across all five quintiles was of borderline significance ($P = 0.055$), whereas when cystatin C was examined as a continuous variable, it was highly statistically significant ($P < 0.001$) No change in the effect size for cystatin C was observed when serum creatinine (or ACR) was substituted for eGFR.

To investigate possible intervening mechanisms, we examined the association between cystatin C, serum creatinine, urinary ACR, and several biomarkers of inflammation (hs-CRP and IL-6) and endothelial dysfunction (E-selectin and sICAM-1) among the control group. Cystatin C was significantly, although modestly, associated with hs-CRP ($r_s = 0.196$), E-selectin ($r_s = 0.179$), sICAM-1 ($r_s = 0.294$), and IL-6 ($P < 0.01$ for each). Cystatin C was not significantly correlated with ACR ($r_s = -0.007$) but was significantly correlated with serum creatinine ($r_s = 0.429$; $P < 0.001$) and eGFR ($r_s = -0.384$; $P < 0.01$). Secondary analyses were performed in which biomarkers of inflammation or endothelial dysfunction were each considered one by one. Neither adjustment for E-selectin nor for sICAM-1 had virtually any effect on the results. Consideration of hs-CRP, IL-6, or eGFR likewise failed to materially alter the original findings.

CONCLUSIONS — In this investigation, we found an approximate threefold increased risk of progression to pre-diabetes among those in the highest quin-

Table 2—*Age-adjusted ORs (95% CI) for progression to pre-diabetes according to selected baseline characteristics*

	Case patients	Control subject	OR (95% CI)
BMI (kg/m^2)			
<27	43 (47.8)	161 (60.3)	1.00
≥27	47 (52.2)	106 (39.7)	1.62 (0.99–2.66)
Family history of diabetes			
No	48 (57.1)	189 (73.3)	1.00
Yes	36 (42.9)	69 (26.7)	1.85 (1.12–3.07)*
Smoking			
Never	34 (37.4)	141 (51.8)	1.00
Ever	57 (62.6)	131 (48.2)	1.95 (1.15–3.31)*
Current alcohol drinker			
No	35 (38.5)	81 (30.1)	1.00
Yes	56 (61.5)	188 (69.9)	0.65 (0.38–1.12)
Hypertension			
No	54 (61.4)	213 (79.2)	1.00
Yes	34 (38.6)	56 (20.8)	2.39 (1.35–4.22)†
Physical activity (total METs in past 7 days)			
< median	52 (57.1)	136 (50.0)	1.00
≥ median	39 (42.9)	136 (50.0)	0.82 (0.40–1.37)
HOMA-IR			
1 (low)	15 (17.0)	90 (33.2)	1.00
2	35 (39.8)	91 (32.6)	2.19 (1.12–4.28)*
3 (high)	38 (42.2)	90 (33.2)	2.75 (1.37–5.54)†
ACR (mg/g)			
<20	78 (92.9)	232 (94.3)	1.00
≥20	6 (7.1)	14 (5.7)	0.94 (0.34–2.60)
eGFR			
< median	52 (57.1)	134 (49.1)	1.00
≥ median	39 (42.9)	139 (50.9)	0.84 (0.50–1.40)
Serum creatinine (g/l)			
< median	39 (42.9)	130 (47.6)	1.00 (0.66–2.11)
≥ median	52 (57.1)	143 (52.4)	1.18

Data are *n* (%) or ORs (95% CI) from conditional logistic regression adjusted for age. Case-patients and control subjects matched on year of baseline interview, sex, race, and year of study enrollment. *$P < 0.05$; †$P < 0.01$.

tile of cystatin C in a population free of diabetes and known CVD. This association was independent of obesity, baseline glucose, eGFR (or serum creatinine), ACR, and other explanatory variables. These results extend previous observations for CVD (17,18,20,32,33) to include the early diabetic stage.

Several studies have examined proteinuria or microalbuminuria in relation to future health events in populations with type 1 diabetes or type 2 diabetes and apparently healthy subjects. Proteinuria is a risk factor for end-stage renal disease among those with type 1 diabetes, and microalbuminuria has been associated with increased CVD mortality among both nondiabetic and type 2 diabetic individuals (10,34–36). The level of cystatin C is also raised in the diabetic state, but this has been related to an increased risk of mortality independent of diabetic status (18).

The results of this study support the hypothesis that cystatin C may be elevated in advance of the onset of clinical diabetes and further suggest a preclinical stage of renal impairment that presages or develops in parallel with the pre-diabetic condition (37). Our results also document the association between cystatin C and pre-diabetes in the context of other risk factors for hyperglycemia and indicate that cystatin C displayed a stronger association than the traditional measures of serum creatinine or the ACR in a population with modest renal impairment These findings suggest that cystatin C may be a more useful marker of renal impairment in populations with mild renal impairment.

The prevalence of hypertension at baseline was higher among the future case patient group than among the matched control subjects. We do not know whether blood pressure rose before or concomitantly with cystatin C levels. Cigarette smoking and intensity of smoking were also higher among case patients than among control subjects. Smoking has been identified in some studies as a predictor of microalbuminuria among individuals with type 1 diabetes (34). Hyperinsulinemia/insulin resistance has also been associated with albuminuria in several reports (10,35,36). Our results confirmed these associations but suggest further that the effect of cystatin C on risk of progression was largely independent of hypertension, smoking, and HOMA-IR.

Preclinical kidney disease has been suggested to reflect mild kidney disease that may increase the risk for subsequent CVD and total mortality; however, the mechanisms remain unclear (37). Our data indicated that cystatin C was significantly correlated with several markers of inflammation including hs-CRP and IL-6. However, in secondary analyses in which these biomarkers were considered, the results remained unaltered. The same held true for markers of endothelial function (E-selectin and sICAM [data not shown]). These results are consistent with findings

Table 3—*Multivariate ORs (95% CI) for progression to pre-diabetes according to baseline quintile of cystatin C (top 20% vs. lowest 80%)*

Cystatin C (mg/l)	Case patients	Control subjects	Model 1	Model 2
Lowest 80%	42 (46.7)	218 (79.9)	1.00	1.00
Top 20%	48 (53.3)	55 (20.1)	5.08 (2.69–9.58)*	3.28 (1.43–7.54)†

Data are *n* (%) or OR (95% CI) from conditional logistic regression using matched cases and controls. All models are conditioned on sex, race, and year of study enrollment. Model 1 was adjusted for age. Model 2 was adjusted for age, BMI (weight in kilograms divided by the square of height in meters), family history of diabetes (no or yes), current smoker (never or ever), lifetime pack-years, fasting glucose (milligrams per deciliter), current drinker (no or yes), baseline hypertension (no or yes), HOMA-IR, and eGFR. *$P < 0.001$; †$P < 0.01$.

from the Cardiovascular Health Study (38) and suggest that the effect of cystatin C on risk of progression to pre-diabetes cannot be solely explained by inflammation or endothelial dysfunction. We did not find an association between urinary ACR and cystatin C, probably because of the narrow distribution of ACR in our study population and/or the use of a single casual urine sample.

Several limitations deserve comment. We did not use an oral glucose tolerance test; thus, some of the study participants classified as pre-diabetic case patients at the follow-up examination may have undetected type 2 diabetes (39). However, fasting glucose measures are more highly correlated over time than the 2-h post-challenge glucose level, and, upon repeat testing, many with newly detected type 2 diabetes are found to "revert" to either impaired glucose tolerance (IGT) or normal (40–42). The Hoorn Study has reported that the risk for conversion to diabetes over 6.5 years is nearly identical among those with impaired fasting glucose (IFG) (51.4/1,000 person-years) and those with IGT (57.9/1,000 person-years), although they are phenotypically distinct groups (43). There are few extant studies that provide data on conversion rates from IFG to pre-diabetes. We also had only single baseline measures of both cystatin C and glucose, which may have introduced random misclassification and would tend to bias our results toward the null. The strengths of the study include the selection of a community-based population, detailed measures of several important covariates, and the assessment of possible intermediate factors including hypertension, smoking, insulin resistance, inflammation, and endothelial dysfunction. Our results, however, need to be replicated, particularly among minority populations. Although observational studies cannot prove causality, the magnitude of the effect size and statistical control for many covariates make our findings compelling and important.

With the advent of "pre-diabetes," the issue of screening and primary prevention is of major public health importance. Since the publication of the results of the Diabetes Prevention Program (DPP), discussion on whether IGT/IFG, although not a disease in itself, may deserve consideration for pharmacological therapy has been increasing (44). There are several arguments against such an approach, including the ineffectiveness of metformin among DPP participants aged ≥60 years. There are also few data on the long-term use of metformin in nondiabetic individuals compared with weight loss and physical activity. The incidence of diabetes after the discontinuation of drug therapy is not known.

Furthermore, the cost-benefit of "medicalizing" a large segment of the population who would need to be monitored for side effects and dose adjustments is unlikely to be favorable. The lifestyle modifications of modest weight loss and increased physical activity used so successfully in the DPP should be considered for those with pre-diabetes (3).

Cystatin C has been shown recently to predict incident coronary heart disease events (45), suggesting that the renal/heart disease connection may share common mechanisms. Our findings provide an important new avenue for future research, suggesting that mild renal impairment may occur early in the natural history of diabetes. Whether cystatin C may also predict type 2 diabetes remains to be tested; but if this theory is proved to be correct, intriguing possibilities are raised for prevention and treatment as it might be fruitful to look for markers of renal impairment early in the course of hyperglycemia that could be modified by lifestyle and hygienic measures.

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