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Research Article

Area Under the Receiver–Operating Characteristics as a Model for Evaluating and Predicting Biomarkers of Early Renal Tubular Damage in Subjects Occupationally Exposed to Lead

Omotosho, I.O.

Department of Chemical Pathology, College of Medicine, University of Ibadan, Ibadan, Nigeria

ABSTRACT

Incidence of kidney failure is on the increase, unfortunately, traditional renal function markers are equivocal especially at the early stage until end-stage renal disease when kidney transplant becomes inevitable. Hence, the need for early and more sensitive marker of renal damage indicating the presence of covert renal damage in occupational lead toxicity is imperative. This work is proposing diagnostic methods that could predict the development of Chronic Renal Failure (CRF) especially in occupational lead-exposed subjects combining results of conventional and new biomarkers of kidney damage using a mathematical model based on Area under the Receiver Operating Characteristics (AUROC). Traditional Renal Function markers (TRF) (plasma creatinine, urea and uric acid) were determined in one hundred each of Lead Exposed Subjects (LES) and non-exposed, non-nephrotic adults (control) along with sixty Chronic Renal Failure patients (CRF) (all age-matched) using standard spectrophotometric methods. Blood lead level (Pb) was determined in all participants using Atomic Absorption Spectrophotometry (AAS) while levels of novel urinary renal enzymes - Glutathione-S-transferase (GST) and N-acetyl-β-D-glucosaminidase (NAG)- activities were also evaluated using ELISA techniques. Pb was used as True Positive Indices (TPI) and TRF along with NAG and GST were used as False Negative Indices (FNI). Ratios of mean, Creatinine: GST (A) (0.01, 0.02 and 0.09), Creatinine:NAG (B) (0.03, 0.08 and 0.6), Uric acid:GST (C) (0.05, 0.08 and 0.08), Uric acid:NAG (D) (0.29, 0.3 and 0.55), Urea:GST (E) (0.17, 0.55 and 0.93), Lead:GST (F) (0.42, 0.59 and 0.88), Lead:NAG (G) (2.56, 2.28 and 6.09), Lead:Creatinine (H) (80.62, 30.37 and 10.22), Lead:Urea (I) (2.46, 1.07 and 0.95) and Lead:Uric acid (J) (8.66, 7.61 and 11.12) for LES, control and CRF groups respectively were computed and used to plot an ROC curve using the FNI values as the abscissa and the TPI values as the ordinate while their AUC were calculated. The AUC values for Lead:Creatinine, Lead:Urea and Lead:Uric acid were 1.00, 0.917 and 0.833 respectively. It is suggested that application of this model after proper standardization may be useful in early identification of covert kidney damage especially in occupationally vulnerable group

Keywords: Area Under Receiver Operating Characteristics (AUROC), Urinary markers of kidney damage, conventional markers of kidney damage, Lead-exposed subjects, chronic renal failure subjects

*Author for correspondence: *E-mail*: *ishiaqomotosh@yahoo.co.uk ;* Tel. +2348023342999

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INTRODUCTION

Lead has been known as an environmental contaminant for several years (Hippocrates in 1841). There have been many reports on its toxic effect on virtually every human system; its effect on the renal system has been among those that have been extensively studied (Markowitz, 2000). In spite of all these and the advancement of medical science even in the developed economies, establishing lead toxicity in the kidney at the early stage to prevent the problem of kidney replacement remains a medical challenge (Edward Lock 2010). Several hypotheses on identifying an early sensitive biological marker of lead toxicity have been equivocal (Poonam and Farhat, 2005; Weeden *et al*, 1975). Several biomarkers of exposure, effect and susceptibility have been proposed, however, problems of sensitivity and specificity especially in cases of continuous low dose exposure characteristic of occupational lead exposure remains a challenge. Blood lead level remains the most promising as a biomarker of exposure which will directly reveal the level of the causative agent of toxicity

(Lock, 2010; Sakai, 2000). However, as promising as this is, the set-backs associated with lead estimation as an index of toxicity which include problems with its estimation and inability to reflect the overall body burden remain issues of importance in its use. Also, the innocuous damage it causes at even sub-lethal doses has made fixing a definite safe reference value a big problem in its application as the gold standard for lead toxicity. In spite of all these draw backs, estimation of blood lead level remains the most plausible gold standard in the diagnosis of lead poisoning including lead nephropathy.

The use of urinary enzymes (NAG and GST) as disease markers is well established (Khalil Manesh *et al*, 1994; Mutti 1989); again, their genetic susceptibility in their use as differential markers for early kidney damage has cast some shadows on their acceptability as reliable markers of kidney damage. Other markers like urinary copro/uroporphyrin, the traditional renal function markers (plasma creatinine, urea and uric acid) (El Saleh, 1993) and even application of Kidney Injury Molecule (KIM) protein are either overtly sensitive or non-specific to be totally reliable (Boventre, 2009). A number of inconsistencies have also been reported as to the universal applicability of single tests in establishing early kidney disease (Siew *et al*, 2011). Hence, the need for a paradigm shift towards developing a diagnostic model that may address this problem.

Area Under the Receiver Operating Characteristic Curve (AUROC) is a well-known measure of ranking performance, estimating the probability that a random positive is ranked before a random negative. It is also often used as a measure of aggregated classification performance, on the grounds that AUC in some sense averages over all possible decision thresholds (Flach *et al*, 2011). Receiver-operating characteristic (ROC) analysis was originally developed during World War II to analyse classification accuracy in differentiating signal from noise in radar detection (Lusted, 1971). Recently, the methodology has been adapted to several clinical areas heavily dependent on screening and diagnostic tests (Zhou, Obuchowski and McClish, 2002; Pepe, 2003). In particular, ROC analysis has been applied in laboratory testing (Campbell, 1994), epidemiology (Shapiro, 1999), radiology (Obuchwski, 2003) and bioinformatics (Lasko *et al*, 2005). ROC analysis is a useful tool for evaluating the performance of diagnostic tests and more generally for evaluating the accuracy of a statistical model (e.g., logistic regression, linear discriminant analysis) that classifies subjects into 1 of 2 categories, diseased or non-diseased. Its function as a simple graphical tool for displaying the accuracy of a medical diagnostic test is one of the most well-known applications. Over the years, application of ROC analysis in evaluating performance of diagnostic tests towards classifying subjects into two classes of "diseased" and "non-diseased" is not new in medical diagnostics (Zou *et al*, 2007,; Tom Fawcett, 2005). This method classically employs the basic rule of sensitivity and specificity of a method in accurately predicting the outcome of an event. In practise, sensitivity is known to be inversely proportional to specificity; thus the method (or combination of methods) that gives the highest specificity (closest fraction of 1) with a sensitivity appropriately moving towards 1 will be the most suitable. It is then mathematically chosen as the most likely method (methods) that will clearly distinguish diseased from non-diseased state even when other results or indicators look apparently normal or contrary (Zou *et al*, 2007).

Consideration of this method as an option in identifying early kidney disease in lead exposure becomes imperative in the absence of a reliable sensitive and specific marker of kidney damage especially in occupationally vulnerable group. This report is thus proposing a mathematical model based on Area Under the Curve of binary results for the various markers of lead toxicity using the ROC with significant p-values for lead exposed subjects using patients already diagnosed with CKD and participants with normal renal function as positive and negative controls respectively. The choice of a diagnostic method will therefore be determined by an AUC with the highest specificity (closest to 1) yet exhibiting very good sensitivity

MATERIALS AND METHODS

This study determined levels of both conventional (plasma creatinine, urea and uric acid) and new [urinary N-acetyl-β-D-glucosaminidase (NAG) and glutathione-S-transferase (GST)] kidney function markers in comparison to the level of conventional biomarker of exposure (blood lead- gold standard) in occupationally exposed participants (cases), established chronic renal failure patients under treatment (positive controls) and participants with normal kidney function clinically assessed (normal control). Results obtained were then computed as binary figures to plot ROC curve the area of which were compared and analyzed statistically to determine which results or result-combinations gave the best in terms of sensitivity and specificity.

Materials

Participants for this study were recruited in three categories as follows:

Normal Control: This group consisted of 50 healthy adults (men and women aged 20-50years) with no kidney disease; they were civil and public servants in and around the University College Hospital, Ibadan, Nigeria. They were selected based on clinical and radiological examination and also on the biochemical indices of kidney functions which were normal in all of them.

Exposed participants: 100 participants (aged 23- 47years) consisting of workers in a lead smelting and battery manufacturing plant (Associated Battery Manufacturing Company (ABM) and ABM Metal Recovery Division based in Ikeja, Lagos) were recruited for this group. Others in the group were those occupationally exposed to lead like automobile-mechanics, battery-repairers, welders, vulcanizers, and vehicle-painters. They have all been exposed to lead occupationally for periods ranging from 3-7years

Positive controls.: 25 CRF patients (aged 35-65years) clinically diagnosed at the medical out-patient department of the University College Hospital, Ibadan were recruited for this group; 20 of these patients were already slated for renal dialysis.

Collection of Samples: 10mls of blood was collected from each of the above participants into lithium heparin bottles. Blood in lithium heparin bottle was divided into two portions; the first portion was used for lead analysis. Plasma was separated from the second portion and analyzed for plasma creatinine, urea and uric acid concentrations, respectively.

Random urine specimens were also collected from participants in the three groups to be used in the determination of urinary microalbumin qualitatively, urinary GST and NAG quantitatively.

Analytical Procedures: Blood Lead (Pb) Assay: This was done using Atomic Absorption Spectrophotometry based on the modified method of Hessel (1968). Blood samples were wet digested and analyzed using Buck Scientific Atomic Absorption Spectrophotometer Model 210VGP (USA). Plasma creatinine concentrations were determined spectrophotometrically based on alkaline picrate method of Jaffe and Larsen as modified by Bartels (1971). Plasma urea was determined spectrophotometrically using the Diacetyl monoxide method of Varley (1969). Plasma uric acid was estimated spectrophotometrically using the ultra violet method of Praetorius *et al* (1972)**.** N- acetyl- β -D-glucosaminidase activity was determined using PPR Diagnostic NAG kit Manufactured by PPR Diagnostics Ltd, England. Urinary Glutamyl-S-Transferase activity was determined using Biotrin GST kit (purchased from Biotrin International Ltd, Dublin, Ireland). This kit is based on Enzyme Immunoassay technique.

The AUROC computation: The above analytical parameters were categorized into two main groups, the true positive and the false negative groups. The true positive was the marker of exposure which undoubtedly was the blood lead (Pb) level; this is the gold standard used. The false negative were levels of other biomarkers of effect including all the conventional kidney function markers (plasma creatinine, urea and uric acid) and the new urinary kidney function markers (NAG and GST). These results were then paired and compared across the three group of participants.

The sensitivity of these results (as binary figures) was evaluated within the 95% CI. The values obtained were then used to plot a ROC curve with the true positive values as the ordinate and the false negative values as the abscissa. The metrics of this curve is the Area under the curve or the AUROC.

These AUROC were then subjected to statistical analysis using ANOVA and Pearson Correlation to determine their sensitivity, specificity and variation towards identifying the most suitable biomarker or combination of biomarkers that may predict covert kidney damage.

RESULTS

Table 1 shows the mean result of lead: 23.4±9.7µg/100ml, 31.7±7.7µg/100ml and 89.9±13.9 µg/100ml in controls, cases and CRF participants respectively (Fig. 1).

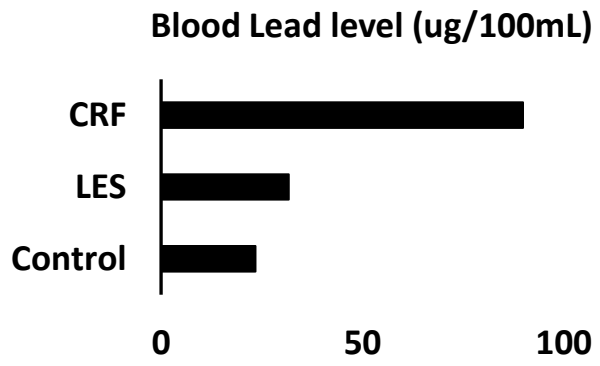

Figure 1
Blood lead levels in control, Chronic Renal Failure (CRF) and Lead exposed (LES) participants.

Table 1:
Creatinine, uric acid, Urea, urinary NAG and GST concentrations in the 3 groups of subjects studied

	Control	LES	CRF
Plasma Creatinine (mg/100ml)	0.77±0.39	0.39±0.45	8.79±5.20
Plasma Uric/Acid (mg/100ml)	3.08±1.10	3.66±1.80	8.08±3.80
Urinary GST (µg/L)	39.47±25.90	75.91±27.70	102.16±54.50
Urinary NAG (IU/L	10.26±1.75	12.39±3.81	14.77±6.27
Plasma Urea (mg/100ml)	21.88±5.03	12.90±7.95	94.91±43.87

Table 2.
Descriptive ratios of Pb blood Pb, plasma creatinine, plasma uric acid, plasma urea, urinary NAG and GST concentrations in the 3 groups of subjects studied

Test Result Variable(s)	Area	S.E[a]	Asymptotic Sig.[b]	Asymptotic 95% Conf. Interval	
				Lower bound	Upper bound
Creat_GST	.000	.000	0.004	.000	.000
Creat_NAG	.000	.000	0.004	.000	.000
Creat_Pb	.000	.000	0.004	.000	.000
UricA_GST	.104	.082	.021	.000	.265
UricA_NAG	.146	.096	.039	.000	.334
UricA_Pb	.146	.099	.039	.000	.339
Urea_GST	.063	.065	.011	.000	.190
Urea_NAG	.083	.080	.015	.000	.240
Urea_Pb	.083	.148	.015	.000	.240
Pb_GST	.604	.121	.544	.313	.895
Pb_NAG	.750	.099	.146	.512	.988
Pb_UricA	.854	.000	.039	.661	1.000
Pb_Creat	1.00	.080	.004	1.000	1.000
Pb_Urea	.917		.015	.760	1.000

a. Under the nonparametric assumption
b. Null hypothesis: true area = 0.5

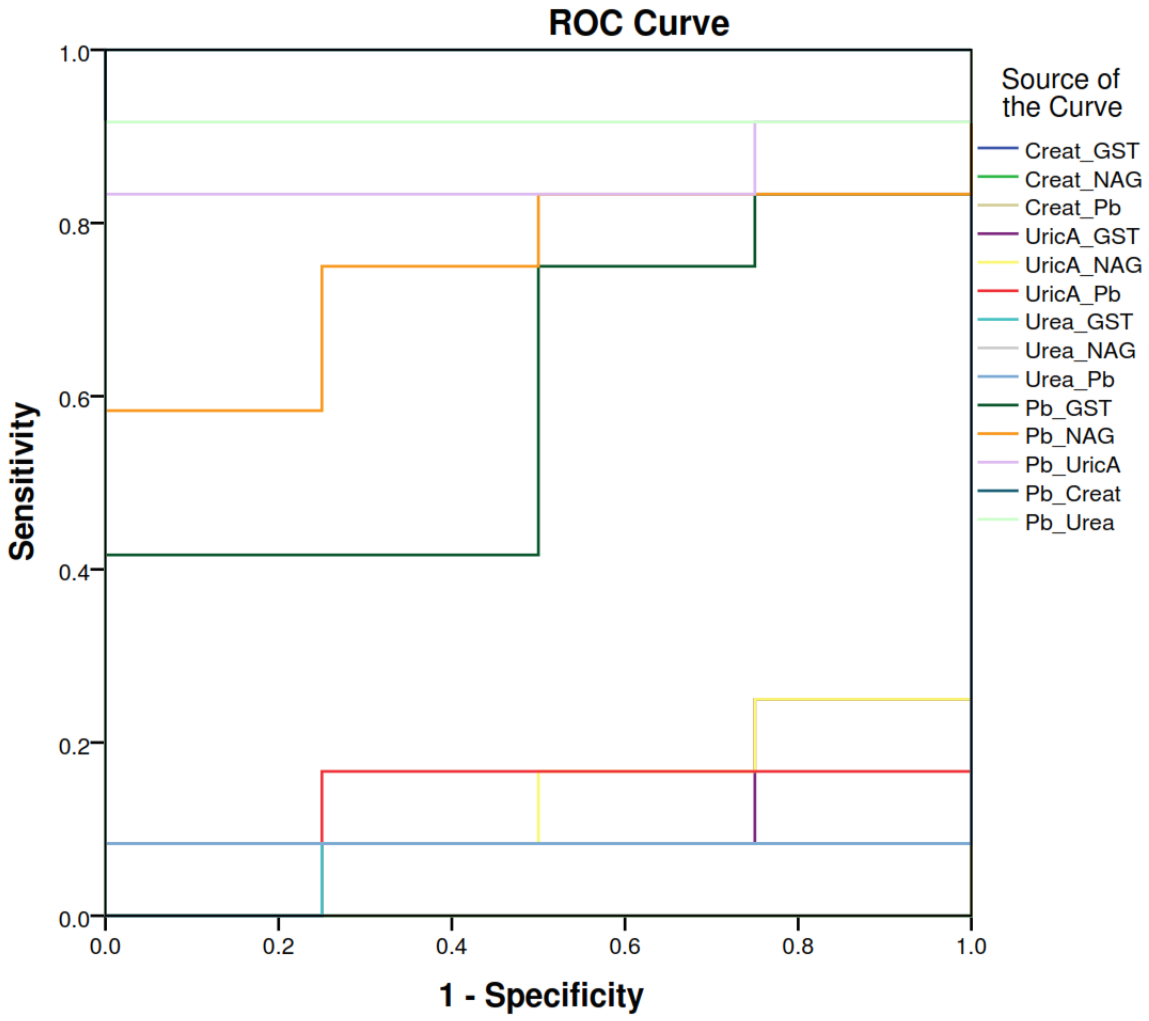

Figure 2:
ROC Curve showing sensitivity, specificity and variation of biomarkers of kidney damage in the three groups of participants

The blood lead level in cases may be said to have indicated lead as the biomarker of exposure and as a true positive indicator of exposure to this toxic metal in these occupationally exposed participants.

Results of plasma creatinine (0.8, 0.4 and 8.8mg/100ml), urea (21.9, 12.9 and 94.9mg/100ml) and uric acid (3.1, 3.7 and 8.1mg/100ml) respectively in controls, cases and CRF participants also indicated non-concomitant elevation in these biomarkers of effect as against values obtained in the biomarker of exposure. This underscores these biomarkers as possible false negative indicators of exposure.

Results of urinary markers NAG (10.3U/L,12.4U/L and 14.8U/L) and GST (3.9ug/L, 75.9ug/L and 102.2ug/L) in control, cases and in CRF participants were equally not clearly indicative as biomarkers of effect in establishing covert lead toxicity in the exposed participants.

Table 2 shows the calculated Mean area of the ratios, their standard error and significance along with their lower and upper limits. The closer the figure was to 1 (one), the greater its positivity and hence the possibility of its application as a biomarker and prognostic value in predicting development of CRF in the subjects especially in those occupationally exposed to Pb.

DISCUSSION

Lead nephropathy is one of the diseases associated with lead toxicity, mortality due to this condition is largely due to the derangement in kidney function especially at the tubular level where the normal tubular secretion and reabsorption of substances is impaired. As old as the problem of lead toxicity is and in as much as there are many research publications on the medical problem, identifying covert lead toxicity at the early stage to allow for necessary intervention remains a medical problem. This work thus set out to investigate whether biomarker of exposure (blood lead level) actually correlated with biomarker of effects [conventional and novel (urinary) kidney function markers] especially at the early stage in occupationally exposed subjects and design a mathematical model that can predict advent of this problem before a

permanent damage is done. The need for a mathematical model was informed not only by the diffuse nature of the problem but also by current trends in biochemical research whereby emphasis is now on in-silico rather than on animal models in research.

In this study, in spite of long term of exposure to the toxic metal (Pb) as aptly demonstrated by increased level of biomarker of exposure (Pb) observed in cases, presence of insidious damage to the kidney by a concomitant increase in the level of biomarker of effect (plasma creatinine, urea and uric acid) levels was not shown. Levels of biomarkers of effect monitored in the exposed participants were all normal except for level of the urinary enzyme, GST, which was raised. The non-concomitant increase in levels of biomarker of effect in lead exposed participants in this study is the general picture peculiar to most people occupationally exposed to low continuous dose of lead (like in lead smelters, welders, vulcanizers etc). Hence, the insidious damage to the kidney due to the continuous exposure may remain unnoticed till end stage renal disease when irreversible damage of the kidney would have occurred. That this insidious damage to the kidney remain unnoticed may not be unconnected with the large reserve capacity of the kidney informed by the large number of nephrons constituting the kidney (Flora et al, 2012). It's been severally reported that clinical and biochemical symptoms of kidney damage may not manifest until more than 60% of the nephrons is destroyed (Flora et al, 2012), hence, effect of exposure to the toxic metal (Pb) may not manifest in the biomarkers of effect as seen in this study until a gross damage has occurred in the kidney. This was clearly demonstrated in all the CRF cases where levels of biomarkers of effect determined in the group were all abnormally high. In contrast to this, there was no corresponding or equivalent increase between the biomarker of exposure (Pb) and biomarkers of effect [true renal failure (TRF)], even the observed increase in urinary GST was not commensurate with the level and years of exposure to the nephrotoxin (Pb). The equally prognostic saturnine gout symptomatic of long exposure to Pb was also not seen in exposed participants in this study.

It was this diffuse picture of chronic exposure to Pb which was not equivalently reflected in the biomarker of effects (conventional and novel kidney damage indicators) that prompted the need to look for a mathematical model that may solve the riddles of early and sensitive diagnosis of lead nephropathy. Thus, Area Under the Receiver Operating Curve (AUROC) was investigated as a possible mathematical tool.

The area under the ROC curve (AUC) is a well-known measure of ranking performance, estimating the probability that a random positive is ranked before a random negative, without committing to a decision threshold. It is also often used as a measure of aggregated classification performance, on the grounds that AUC in some sense averages over all possible decision thresholds that would not only identify a more selective and specific biomarker but would also be prognostic of progression to end stage renal disease. The veracity of ROC as a tool for verifying a method and in predicting an event lies on the ability to make a binary prediction which can be in 4 different outcomes (Metz,1978): (a) A True Negative, i.e. this correctly predict that the class is negative (0.0) (b: A False Negative, i.e. this incorrectly predict that the class is negative (c): A False Positive, i.e. we incorrectly predict that the class is positive (d): A True Positive, i.e. we correctly predict that the class is positive (1.0).

Hence, combining levels of biomarker of exposure (Pb) with those of effects (TRF) and expressing the binomial values of these as coordinates on the ROC revealed a distinct profile of the relationship between the two sets of biomarkers. The distinct picture was better seen in the combination of Pb vs Creatinine, Pb vs Urea and Pb vs Uric acid in comparison to the combination of Pb vs GST and Pb vs NAG. Hence, the calculated AUROC was closer to the true value of 1.0 in the earlier combination than the latter. It may be inferred that combining the values by expressing levels of biomarker of exposure (Pb) as the ratio of TRF as stated above may be similar to determining the clearance/excretory rate of Pb relative to those of TRF.

Therefore, the relatively better prognostic picture of kidney damage as seen in the higher AUROC values of Pb vs TRF in comparison to those of urinary enzyme markers may be indicative of the insidious damage which ordinarily would not be noticed until a later stage of continuous exposure to the nephrotoxin (Pb). At that stage, the damage would have become irreversible.

Mathematically, for a ratio (x/y) to be tending towards 1.0, the denominator (y) must be on the increase almost in the same proportion with the nominator. Hence physiologically, a hypothetic situation will be reached where the rate of accumulation of x (which in this instance is the biomarker of exposure (Pb) would have precipitated so much of the TRF (biomarker of effect) to the extent of the ratio reaching the true value of 1.0. The lower ratios of AUROC of Pb vs NAG and GST may thus indicate a better clearance/excretory rate of Pb relative to those of the TRF in chronic Pb exposure. In acute exposure, the levels of urinary NAG and GST will be high enough to be prognostic of acute renal failure which may be due to secretion of the two enzymes (directly in the renal tubular cells) (Wiland et al, 1997) and their faster rate of clearance (tubular excretion) as different from the TRF which are more indicative of renal glomerular function (Bulent et al, 2016).

In conclusion, a mathematical model expressing levels of Pb (as the biomarker of exposure) against the levels of the TRF and calculating the AUROC from the ROC of their binomial values may give a better and early prognostic picture of the development of chronic kidney failure especially in subjects chronically exposed to Pb as in those occupationally routinely exposed after proper standardization is hereby proposed. Drawing up of a normogram based on this model may also be a plausible approach to applying this model..

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