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RESEARCH ARTICLE

PCT discriminates aerobic and anaerobic bacteremia from nonbacterial blood infections: A 10 years study in CKD patients.

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Abstract

Background: Bacteremia can sometimes be difficult to substantiate. Its early distinction from non-bacterial blood stream infections in Chronic Kidney Disease patients is often a challenge due to functional deterioration of various components of immune system. Serum Procalcitonin assay is one of the biomarkers of sepsis. Thus the aim of this study is to analyze the usefulness of Procalcitonin as both diagnostic and prognostic marker for aerobic and anaerobic bacteremia and to assess the correlation between serum Procalcitonin and time to positivity.

Methods: The study is based on the analysis of our data over a period of 10 years. We retrospectively analyzed 1168 Chronic Kidney Disease patients with suspected blood stream infections who had concurrent Procalcitonin data and blood culture results.

Results: Procalcitonin levels were significantly correlated with Time to Positivity and also with survival of patients in positive blood cultures. Survival analysis revealed that patient with high Procalcitonin had significant low survival rate than patients with low Procalcitonin. Area under the Receiver operative characteristic curve for aerobes, anaerobes, fungus and negative blood cultures was 0.920, 0.913, 0.110 and 0.016 respectively. The optimal cut-off value of serum Procalcitonin for predicting aerobic bacteremia was 1.3 and 1.29 for anaerobic blood stream infections.

Conclusions: Procalcitonin is a reliable and promising biomarker to discriminate aerobic and anaerobic bacteremia from non-bacterial blood stream infections. Procalcitonin may also predict the severity and prognosis of Blood stream infections but renal function should be taken into account.

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Introduction:-

Severe infection and sepsis with accompanying dysfunction or failure of multiple organs are major causes of morbidity and mortality in patients with chronic kidney disease (CKD) (James et al., 2009; Dalrymple et al., 2012). These patients are particularly susceptible to infection because of functional deterioration of many components of the immune system (Girndt et al., 2001). Due to their compromised immune statuses, clinical signs for infection in these patients are often subtle and nonspecific and the conventional laboratory markers are often influenced by uremia (Lu et al., 2013). However, it is difficult to differentiate between infectious causes and non-infectious causes of systemic inflammatory responses because of chronic elevation of inflammatory markers and nonspecific clinical symptoms that are common among patients with CKD (James et al., 2009; Panichi et al., 2001).

Bloodstream infections such as bacteremia and sepsis are potentially life-threatening and thus require early diagnosis and prompt administration of antibiotics to reduce mortality related to multiple organ failure (Nakamura et al., 2009; Jensen et al., 2006). Blood cultures (BCs) are the "gold standard" for diagnosis of sepsis (Weinstein et al., 1997). However, test results are typically not available for 12 to 48 hours therefore a rapid and reliable test to

confirm or exclude the existence of blood-stream infection would thus be very useful when deciding on the need for antibiotics.

Procalcitonin (PCT), a 116-amino acid precursor protein of calcitonin, has been shown to be able to accurately distinguish bacterial from nonbacterial infections, or other sterile inflammation condition (Simon et al., 2004; Tang et al., 2009; Jones et al., 2007). PCT is constitutively produced in the C cells of the thyroid gland without hormone activity. It is rapidly produced and released to peripheral circulation in response to endotoxin and pro-inflammatory cytokines, such as IL-1 β and TNF- α . Unlike CRP, PCT production is inhibited by interferon-gamma (IFN- γ), a cytokine that is produced during viral infection (Guz et al., 2006; Herget-Rosenthal et al., 2001; Lam et al., 2008; Öztürk et al., 2010; Steinbach et al., 2004; Yilmaz et al., 2007; Amour et al., 2008). PCT has not been extensively studied in patients with chronic kidney disease and it was never analyzed with anaerobic bacteremia. Thus the aim of this study is to analyze the usefulness of PCT as both diagnostic and prognostic marker for aerobic and anaerobic bacteremia and to assess the correlation between PCT and time of detection.

Materials and methods:-

We retrospectively analyzed the data of 1168 CKD patients with suspected bloodstream infections who had concurrent Serum PCT data and blood culture results and were admitted from Jan 2006 to mar 2011 at our centre. For each patient age, sex, underlying disease, Serum PCT levels, time of blood culture positivity, results of blood culture and its outcome were recorded.

Blood samples for Serum PCT and blood culture were received at the same time in the microbiology laboratory. Out of 15 ml blood 5 ml was kept for S. PCT while 5 ml blood was collected in FA bottle for the isolation of aerobe and fungus while 5 ml in FN bottle for isolation of anaerobes. These two inoculated bottles were further incubated in BactAlert 3D system following standard protocols until a positive result was obtained or for up to 7 days. Time of positivity was recorded and 0.5 ml blood was drawn from the positive bottles immediately and inoculated onto sheep blood agar, chocolate agar, MacConkey agar, Anaerobic agar plates, Sabourauds agar and were incubated at 37°C. Microorganisms were further identified by Vitek-2 system (BioMerieux, France).

PCT levels were measured in 200 μ l sera according to the manufacturer's instructions via automatic analyser VIDAS B.R.A.H.M.S. (BioMerieux, France). The lower limit of detection of the assay was 0.05 ng/mL

To assess the diagnostic value of PCT for bacteremia, blood cultures were classified into two groups- positive blood culture and negative blood culture. Positive blood cultures were further divided into three categories according to the microorganisms identified: Aerobes, Anaerobes and Fungi for the purpose of determining the usefulness of PCT for early diagnosis of bacteremia. Time to positivity (TTP) was defined as the interval between the start of incubation and detection of growth by the automated blood culture system.

Survival from the date when the blood culture was obtained was investigated from the medical records. This study attempted to estimate the prognosis of survival of patients with Positive bloodstream infection. Follow-up information for at least 30 days was compiled for all patients. If a patient had more than one positive blood culture within 30 days, only the first was considered for survival analysis.

Data were expressed as mean + standard deviation. Statistical analysis was performed using chi square test, contingency coefficient and one-way ANOVA while significance was defined at p value of 0.05. Diagnostic accuracy was assessed by calculating the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Receiver operating characteristic (ROC) curves were drawn and the area under each ROC curve (AUC) was calculated to assess the diagnostic value of PCT, to discriminate bacteremia (i.e., positive blood culture) from fungemia and nonbacteremia (i.e., negative blood culture) The correlation between PCT and TTP was assessed with the Spearman rank test and Kaplan-Meier method was used to estimate survival curves.

Results:-

Blood samples of 1168 CKD patients for PCT and blood cultures were taken simultaneously. Their base line and demographic data were described as follows. Out of 1168 CKD patients males were 43.8% and females were 56.2%, the mean age of the study population was 42.40 + 14.90 and predominant causes of CKD in this group were (46.6%)

diabetic nephropathy, (32.2%) glomerulonephritis, (15.7%) hypertension and (5.5%) unknown. Demographic features and blood culture results are described in Table 1.

Out of 1168 blood samples 984 samples were found positive and remaining 184 samples did not show any growth until 7th day, hence considered as negative. For these positive blood cultures, PCT levels were significantly correlated with TTP at 0.05 levels as depicted in Table 2.

Among 984 (84.3%) monomicrobial BCs, 560 (56.9%) blood cultures were found positive for aerobes, 376 (38.3%) for anaerobes and 48 (4.8%) for fungal pathogens. Among aerobes 12.7% were *Klebsiella aerogenes*, 17.1% *Klebsiella pneumoniae*, 21.1% *Citrobacter freundii*, 11.3% *Enterobacter aerogenes*, 8.4% *Acinetobacter*, 1.3% *Morganella morganii*, 1.3% *Proteus rettgeri*, 7.1% *Proteus vulgaris*, 8.4% *Pseudomonas aeruginosa*, 7.4% *Staphylococcus aureus*, 2.6% *Staphylococcus saprophyticus* and 1.3% *Streptococcus pyogenes* Figure 1(A). Among anaerobes 38.2% were *Bacteroides fragilis*, 8.5% *Bacteroides ovatus*, 29.7% *Fusobacterium nucleatum*, 2.2% *Fusobacterium varium*, 19.2% *Peptostreptococcus anaerobius* and 2.2% *Peptococcus* Figure 2(A) while in fungi 50.0% were *Candida tropicalis*, 33.3% *Candida glabrata* and 16.7% *Candida krusei* Figure 3(A) were isolated. Most frequent aerobe was *Citrobacter freundii* (21.1%), anaerobe was *Bacteroides fragilis* (38.2%) and fungus was *Candida tropicalis* (50.0%). All the Figures are depicted along with their corresponding ROCs. The diagnostic accuracy of PCT for discriminating aerobes, anaerobes, fungus and sterile blood cultures are shown in Figure 1(B), 2(B), 3(B) and 4(A) respectively.

AUC in this ROC plot is 0.920 Figure 1(C) and the optimal cut off value of PCT for predicting aerobic blood stream infection was 1.3 ng/ml. Using this cut off value the sensitivity, the specificity, PPV and NPV were 90.0%, 70.3%, 55.1% and 94.5% respectively. Hence AUC signifies that PCT can very accurately predict the aerobic blood stream infection and proved to be highly sensitive.

AUC in this ROC plot is 0.913 Figure 2(C) and the optimal cut off value of PCT for predicting anaerobic blood stream infection was 1.29 ng/ml. Using this cut off value the sensitivity, the specificity, PPV and NPV were 89.0%, 67.1%, 58.2% and 95.9% respectively. Hence AUC signifies that PCT can very accurately predict the anaerobic blood stream infection and proved to be highly sensitive.

AUC in this ROC plot is 0.110 Figure 3(C) which illustrates that PCT is not useful to detect fungal blood stream infection.

AUC in this ROC plot is 0.016 Figure 4(B) which illustrates that PCT is not useful for Negative blood culture that means there is no blood stream infection.

Analysis between PCT and pathogens were done by one way ANOVA as depicted in Table 3 and results suggest that after comparing means two subsets were found. Sterile blood culture and fungal blood culture formed one subset while aerobic and anaerobic blood stream infections formed another subset. Hence statistical analysis also indicates that PCT levels discriminate bacteremia from nonbacteremia and fungemia.

Our results also demonstrated that PCT was significantly correlated with the survival of patients who had positive blood culture. Figure 5 showed that patient with high PCT levels had a significant low survival rate than those with a low PCT level.

Discussion:-

To our knowledge, this is the first and largest study from India so far to examine the clinical usefulness of PCT in CKD patients with suspected bloodstream infection managed at a single institution. We found that the PCT was rapid, more accurate and useful for predicting bloodstream bacterial infection and its level was significantly high in patients with a positive blood culture. It was also noticed that PCT levels were significantly correlated with TTP.

According to previous studies renal function appears to influence the PCT level (Meisner et al., 2001). The cause of PCT elevation in patients with renal dysfunction could be its impaired renal or hepatic elimination or its increased production. Peripheral blood mononuclear cells release more PCT in patients with impaired renal function and those receiving renal replacement therapy (Herget-Rosenthal et al., 2005). In addition, patients with severe renal dysfunction often show evidence of a systemic inflammatory response, which leads to PCT production (Meisner et al., 2001). Furthermore, PCT was not distinctively elevated in patients with less severe infections, and the basal level of PCT increased in patients with impaired renal function (Herget-Rosenthal et al., 2001; Castelli et al., 2004;

Herget-Rosenthal et al., 2005; Dahaba et al., 2003; Schmidt et al., 2000; Opatrna et al., 2005; Lu et al., 2013) thus, a higher cutoff value for PCT was suggested for use in patients with impaired renal function (Herget-Rosenthal et al., 2001; Dahaba et al., 2003; Lu et al., 2013). Similarly, in our study, the best cut off value of PCT for diagnosing bacterial infection was 1.3 ng/ml with 90.0% sensitivity, 70.3% specificity, 55.1% PPV and 94.5% NPV while for anaerobic bacteremia best cut off value was 1.29 ng/ml with 89.0% sensitivity, 67.1% specificity, 58.2% PPV, and 95.9% NPV for patients with CKD.

Several studies have shown that a short TTP is associated with a significantly high mortality rate in patients with *Staphylococcus aureus* bloodstream infection (Marra et al., 2006; Sowden et al., 2008). In the present study, high PCT values are directly proportional to bacterial load and the severity of infection. As the bacterial load increases, severity of infection will be increased which in turn raises the PCT levels resulting in short TTP that is why PCT and TTP were significantly correlated, suggesting that PCT can be a good prognostic factor for bacteremia. Some authors have provided convincing evidence that PCT is useful not only for detecting bacteremia but also for evaluating severity (predicting mortality) in patients with pneumonia in the ICU patients (Kruger et al., 2008; Schuetz et al., 2011; Huang et al., 2008). Jensen et al (2006) found that a high maximum PCT level and the daily changes of PCT were independent predictors of 90-day mortality in patients in the ICU. The CAPNETZ study reported a high prognostic value of PCT for predicting mortality in patients with CAP (Kruger et al., 2008). In contrast, the GenIMS cohort study only found moderate additional value of PCT compared with the pneumonia severity index and the CURB-65 score (Huang et al., 2008). To the best of our knowledge, however, no previous study from India has reported the PCT as prognostic factor for bacteremia in CKD patient from a single institution. Our results demonstrated that PCT was significantly correlated with the survival of patients who had positive blood cultures. Survival analysis shows that patient with high PCT levels had a significant lower survival rate than those with a low PCT level. Since PCT secretions begin within four hours of stimulation and peaks at 8 hours (Simon et al., 2004) and the assay time for PCT is only about 20 minutes. Hence the availability of its results become much earlier than gold standard blood culture. Thus values of PCT can help physicians to safely withhold higher antibiotics in patients with suspected bloodstream infections or identify high-risk patients, which could have a major impact on clinical practice. Its measurement may aid decision making, particularly in the CKD patients.

Therefore the study illustrates that the PCT is a very reliable biomarker that contributes in the early diagnosis of suspected blood stream aerobic and anaerobic infections and it is also useful to evaluate the severity and prognosis of blood stream infections however, renal function should be taken into account when using this biomarker.

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Table 1:- Demographic features and blood culture results

Demographic Features:	
Age	42.40 + 14.90
Gender	Male 512 (43.8%) Female 656 (56.2%)
Primary Diagnosis:	
Diabetes	544 (46.6%)
Glomerulonephritis	376 (32.2%)
Hypertension	184 (15.7%)
Unknown	64 (5.5%)
Results of Blood Culture:	
Total blood cultures	1168
Positive blood cultures	984 (84.3%)
Aerobes isolated	560 (56.9%)
Anaerobes isolated	376 (38.3%)
Fungus isolated	48 (4.8%)
Negative blood cultures	184 (15.7%)

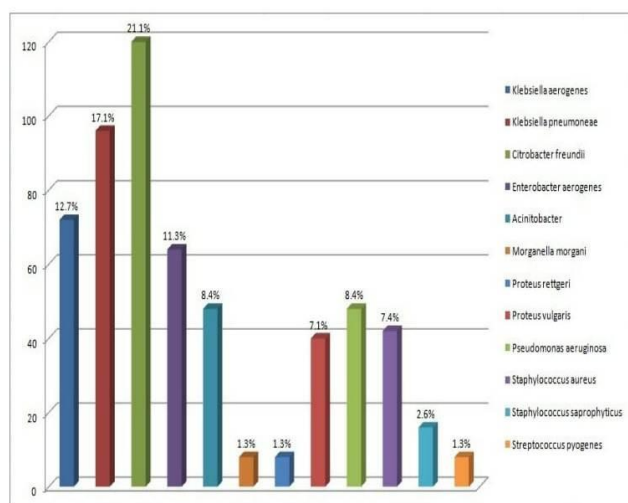
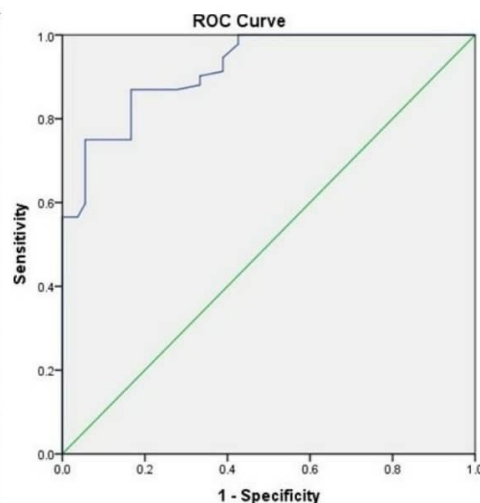
Table 2:- Correlation analysis between PCT and TTP of blood cultures

Spearman's rho		Time of Detection HR	Serum PCT
Time of Detection HR	Correlation coefficient	1.000	0.138*
	Sig. (1-tailed)	0.0	0.049
	N	146	146
Serum PCT	Correlation coefficient	0.138*	1.000
	Sig. (1-tailed)	0.049	0.0
	N	146	146

* Correlation is significant at the 0.05 level (1-tailed).

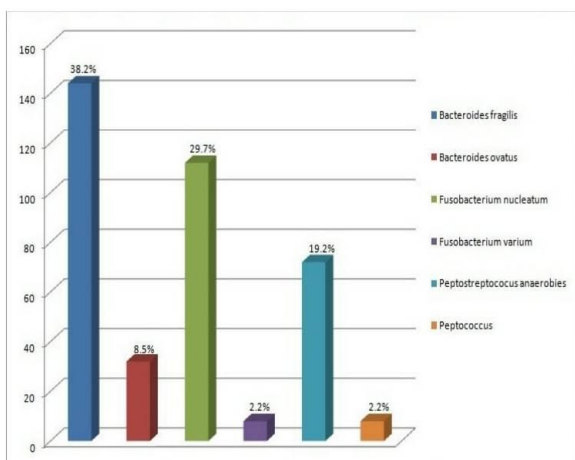
Table 3:- One-way ANOVA analysis to compare means for groups in homogeneous subsets

Pathogen	N	Subset for alpha = 0.05	
		1	2
Negative	184	0.6161	--
Fungus	48	0.9367	--
Anaerobes	376	--	6.3536
Aerobes	560	--	6.6611

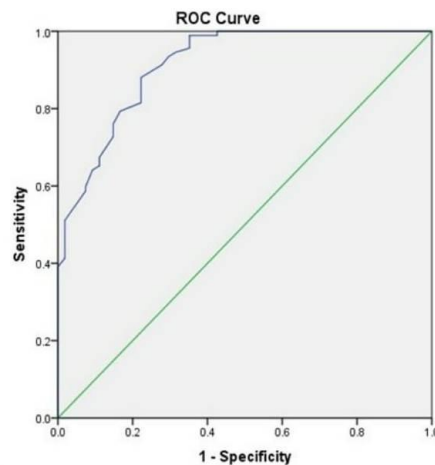
**A:** Spectrum of aerobes in Blood.**B:** Diagonal segments are produced by ties. ROC of PCT for predicting blood stream aerobic infection.

Area	Std. Error ^a	Asymptotic Sig ^b	Asymptotic 95% confidence interval	
			Lower Bound	Upper Bound
0.920	0.021	0.000	0.879	0.962

C: Area under curve**Fig 1:-** (A) Spectrum of aerobes in Blood (B) ROC of aerobes (C) Area under curve for aerobes.



A: Spectrum of anaerobes in Blood.

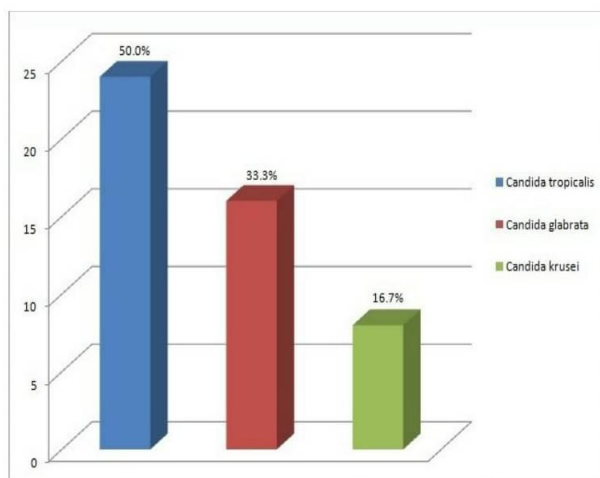


B: Diagonal segments are produced by ties. ROC of PCT for predicting blood stream anaerobic infection.

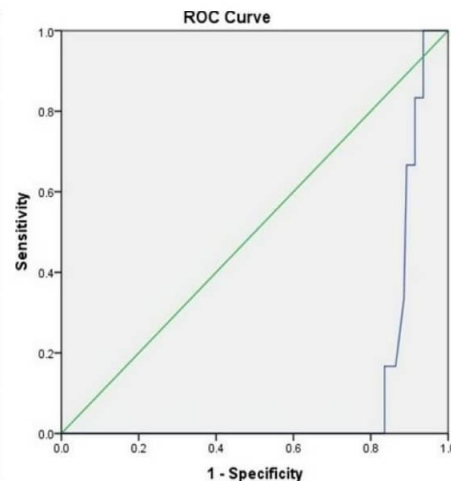
Area	Std. Error ^a	Asymptotic Sig ^b	Asymptotic 95% confidence interval	
			Lower Bound	Upper Bound
0.913	0.024	0.000	0.867	0.960

C: Area under curve

Fig 2:- (A) Spectrum of anaerobes in Blood (B) ROC of anaerobes (C) Area under curve for anaerobes



A: Spectrum of fungus in Blood.

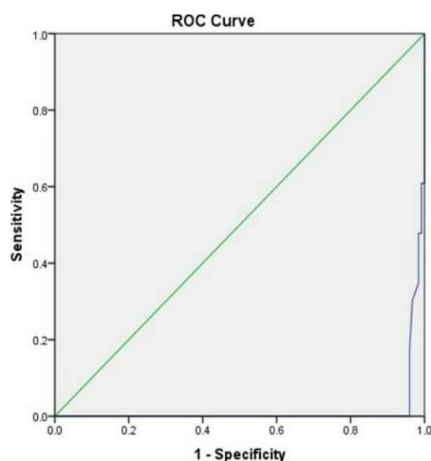


B: Diagonal segments are produced by ties. ROC of PCT for predicting blood stream fungal infection.

Area	Std. Error ^a	Asymptotic Sig ^b	Asymptotic 95% confidence interval	
			Lower Bound	Upper Bound
0.110	0.028	0.001	0.056	0.164

C: Area under curve

Fig 3:- (A) Spectrum of fungi in Blood (B) ROC of fungi (C) Area under curve for fungi



A: Diagonal segments are produced by ties.

ROC of PCT for no bacteraemia

Area	Std. Error ^a	Asymptotic Sig ^b	Asymptotic 95% confidence interval	
			Lower Bound	Upper Bound
0.016	0.008	0.000	0.000	0.33

B: Area under curve

Fig 4 (A) ROC of no bacteremia (B) Area under curve for no bacterial blood stream infection

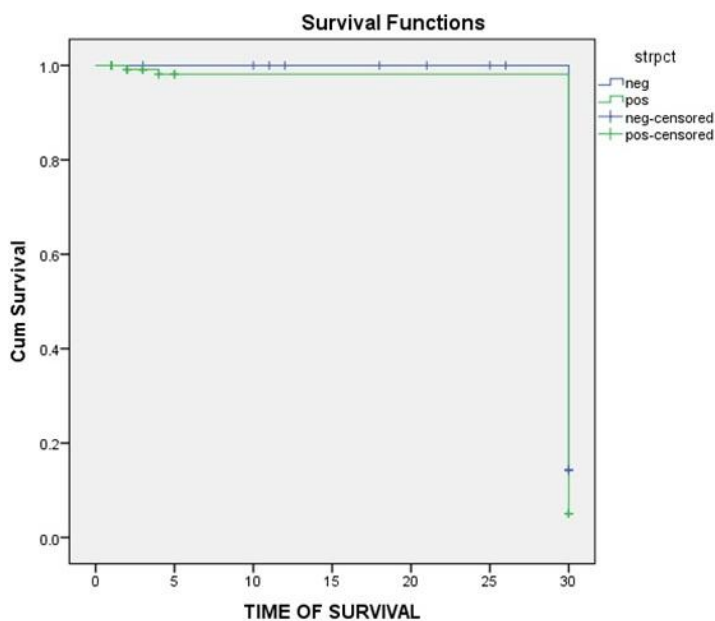


Fig 5:- Kaplan-Meier survival curve of patients with positive blood culture

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