

Original Article

Sweat conductivity test - can it be a cheaper alternative to sweat chloride analysis for diagnosis of cystic fibrosis in low resource setting?

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Background & objectives: Availability of sweat chloride analysis, the gold standard test for diagnosis of Cystic Fibrosis (CF) faces significant challenges in India. This study aimed to compare sweat conductivity using Sweat-Chek™ Sweat Analyzer against sweat chloride analysis using the 926 Sherwood chloride analyser and assess if sweat conductivity test can guide CF diagnosis in resource-poor settings.

Methods: In this retrospective study sweat chloride analysis and sweat conductivity were simultaneously performed on samples collected *via* Macroduct® system from patients referred for sweat testing. CF diagnosis was based on sweat chloride levels: ≥ 60 mmol/l confirmed CF, 30-59 mmol/l was borderline, and < 30 mmol/l excluded CF. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), and area under curve (AUC) were calculated via ROC curve. Spearman's rho was employed to analyse the correlation between methods.

Results: Both tests were performed on 118 children of which 106 samples were adequately collected. CF was diagnosed in 11 children. Sweat conductivity ≥ 80 mmol/l diagnosed CF with 100 per cent sensitivity, specificity, PPV, and NPV. Likewise, a value ≤ 49 mmol/l predicted absence of CF with 100 per cent sensitivity, 91.36 per cent specificity, 78.13 per cent PPV, and 100 per cent NPV. Spearman's rho of 0.93 ($P < 0.001$) showed a strong correlation between the two methods. Intermediate conductivity values also correlated well ($r_s 0.62$, $P < 0.003$) with intermediate sweat chloride levels.

Interpretations & conclusions: Sweat conductivity reliably identified CF in the study population including those children with borderline levels, suggesting the possibility of its use in resource-limited settings where sweat chloride analyzers are unavailable.

Key words Cystic fibrosis - cystic fibrosis transmembrane conductance regulator - nanoduct - pilocarpine nitrate - sweat conductivity - sweat chloride analysis

Cystic fibrosis (CF) is a life-shortening autosomal recessive disease characterised by fat malabsorption, poor growth, and recurrent sino-pulmonary infections.

It is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene located on the long arm of chromosome 7¹⁻³. *CFTR*

protein serves as a chloride channel on the epithelial cell surface. Mutations in the *CFTR* gene result in absent or deficient CFTR protein function, thus causing various organ pathologies. In the sweat ducts, abnormal CFTR protein prevents the reabsorption of chloride and sodium from the secreted sweat as it traverses through the ducts to be excreted on the skin surface⁴. This results in the excretion of sweat with high electrolyte content, the patient, thus facing devastating consequences of dys-electrolytaemia, especially during summers^{5,6}. This observation led to the development of the sweat chloride test by Gibson and Cooke in 1959, and since then it has remained the gold-standard diagnostic test⁷. Values ≥ 60 mmol/l are considered diagnostic of CF.

Existing literature shows that sweat conductivity measurement has a good correlation with sweat chloride analysis^{8,9}. Sweat conductivity test measures the capacity to conduct electrical current through sweat, which mainly depends on the concentration of positive and negative ions, the bulk of which are sodium and chloride ions, respectively. Guidelines by the National Committee for Clinical Laboratory Standards (NCCLS) and the Cystic Fibrosis Foundation (CFF) classify sweat conductivity test as a screening test for CF and recommend that a value more than 50 mmol/l should be confirmed by a quantitative sweat chloride analysis^{10,11}.

Variability in sweat chloride levels across ethnicities is documented, but data on sweat conductivity variability is limited. A few studies have reported a good correlation between the two tests in non-Caucasian groups, though none are from Asia^{8,9}. In India, clinicians often rely on sweat conductivity for CF diagnosis due to its lower cost. In this study, we compared the performance of the sweat conductivity test against sweat chloride analysis by carrying out both tests on the same sweat sample collected by Macroduct[®] Sweat Collection System (Wescor, Logan, UT, USA).

Materials & Methods

This retrospective study analysed CF diagnostic testing data from February 2017 to January 2018 and was conducted at the department of Biochemistry, Christian Medical College and Hospital, Vellore, a tertiary referral centre in Southern India. Institutional review board (IRB) clearance was obtained before data collection, and consent was waived due to the retrospective nature of the study. The objectives of this study were to compare values obtained by sweat

conductivity test using the Sweat Chek system against those by quantitative sweat chloride analysis on sweat samples collected by pilocarpine iontophoresis from study participants from the Indian subcontinent. De-identified data were analysed. We also examined the level of correlation between diagnostic and intermediate-range sweat chloride values with corresponding sweat conductivity values when performed on the same sweat samples.

Inclusion criteria: Participants in whom CF was suspected and who were referred for sweat testing were included if an adequate sweat sample was obtained for both tests.

Study protocol: Up till 2016, diagnosis of CF in our institution was done by sweat conductivity testing. On procuring equipment for quantitative sweat chloride analysis, both tests were done on the same sweat samples for quality check. In the present study, available data on the results of sweat tests by both methods were compared. Sweat induction and collection were done either at the bedside of the patient or in the laboratory (for ambulatory patients), and the collected samples were immediately analysed. Sweat chloride value of ≥ 60 mmol/l was considered as diagnostic of CF.

Procedure of sweat induction for conductivity and sweat chloride analysis: For sweat induction, the skin of the forearm was cleaned with 70 per cent alcohol, wiped with deionized water, and dried completely. Then, iontophoretic stimulation was done using two copper electrodes holding agar disks, containing pilocarpine nitrate solution (0.5%) strapped to the cleaned forearm. The electrodes were connected to a battery-operated current source, which delivers a maximum current of 1.5 mA for 5 min (Supplementary Table). After completion of iontophoresis, the electrodes were removed, and the skin was cleaned with deionised water and dried with a cotton swab without delay. The Macroduct[®] Sweat Collection System (ELITechGroup Inc. Logan, Utah, USA) (Supplementary Table) was then placed over the area where the positive electrode was placed and sweat collected. The collected sweat was then used to perform both assays within 20 min of sample collection.

Principle of conductivity method and procedure: The sweat sample was analysed using 3120 Sweat-Chek[™] Sweat Analyzer (Wescor Inc, Logan. UT, USA) according to the manufacturer's instructions

(Supplementary Table). Quality control (ELITech Group Inc Utah, USA) was run along with the sample analysis. The measuring cell was cleaned thoroughly with deionised water and air-dried after each sample to prevent any carry-over from previous analyses.

After the sweat collection was over, the spiral collection tube was detached from the Macroduct® sweat Collection System and attached to the inlet of the Sweat-Chek™ Sweat Analyzer. It was then injected into the conductivity cell using a clean dry syringe connected to the end of the spiral tube and allowed to stand for a minute for readings to stabilise. The equipment measured the conductivity of the specimen using electrodes and converted it into equivalent sodium chloride molarity, and the result was displayed.

Principle of sweat chloride analysis test and procedure:

The sweat chloride concentration estimation was performed using a 926 Sherwood chloride analyser (Sherwood Scientific Ltd., Cambridge, UK) (Supplementary Table) based on the principle of coulometric titration. Instrument titrates chloride by passing a constant current between two silver electrodes, which generates silver ions. These silver ions combine with the chloride in the sample to form silver chloride in the acid buffer.

The sweat sample collected was transferred to a clean, dry vial, and 20 µl of sweat was pipetted out to the titration vessel containing 14 ml of buffer solution. Once all chloride ions from the sweat sample get used up in the reaction to form silver chloride, free silver ions appear in the solution, causing a sudden change in conductivity. This change is detected by the sensing electrodes and results are displayed directly in millimoles per litre. Once the readout in the instrument is stabilised, results are displayed as mmol/l chloride.

At the end of each test, values of sweat conductivity and sweat chloride analysis were entered in the laboratory register. For the study, the Principal Investigator collected all the test values and relevant demographic and clinical information into a data entry sheet for analysis.

Statistical analysis: The Mean (SD) and Median (range) were reported for continuous data. The number (with percentage) was reported for the categorical data. A box plot was presented to show the distribution of the quantitative sweat chloride level (<30, 30-59, and ≥60 mmol/l). The scatter plot was presented to visually assess the correlation between two

continuous variables. Pearson correlation coefficient (r) was estimated to find the relationship between two continuous variables. Nonparametric Spearman rank correlation was used to assess the correlation between two continuous skewed variables. The receiver operating characteristic (ROC) curve was drawn to find the capability of the sweat conductivity test in discriminating CF from non-CF subjects. Diagnostic statistics such as sensitivity, specificity, positive predictive value, and negative predictive values were reported. All tests were two-sided at $\alpha=0.05$ level of significance. All analyses were done using Statistical Package for Social Sciences (SPSS) software Version 21.0 (IBM Corp, Armonk, NY, USA).

Results

During the study period, 118 study participants underwent sweat testing in our laboratory; 106 of them aged three months to 55 yr (median age of 6 yr) fulfilled the inclusion criteria. Eleven study participants were above the age of 18. A diagnosis of CF was established in 11 (10.4%) study participants based on a cut-off ≥60 mmol/l sweat chloride level measured *via* sweat chloride analysis. Table I gives the clinical characteristics of study participants with CF (pwCF) and those without CF.

A Spearman's rho value of 0.93 ($P<0.001$) was obtained by performing a correlation analysis between the two diagnostic methods. Figures 1A and B show the frequency distribution of sweat conductivity values plotted against the cohort's sweat chloride analysis values.

An ROC curve was constructed to check the capability of the sweat conductivity test in discriminating CF from non-CF. The area under the ROC curve (AUC) for sweat conductivity measurement as a diagnostic test for CF was 1 (Fig. 1C), indicating that this test could perfectly distinguish between individuals with CF and those without. The cut-off >79 mmol/l was the optimal cut-off point for distinguishing between CF positive and negative individuals.

An AUC of 0.988 was obtained under ROC curve plotted to assess the ability for sweat conductivity in separating normal individuals (sweat Chloride level of <30 mmol/l) from those with borderline high sweat chloride levels (30 to 59 mmol/l) (Fig. 1D). The optimal threshold of >49 mmol/l provided perfect sensitivity and high specificity, making it an excellent diagnostic tool to rule out CF in a patient.

Table I. Comparison of demographic and clinical features and results of quantitative sweat chloride analysis and sweat conductivity tests of patients with and without cystic fibrosis

Characteristics	Study participants with CF (n=11), n (%)	Non-CF Study participants (n=95), n (%)		
Age (yr), mean±SD	8.3±15.8	8.5±9.4		
Age categories (yr)				
<2	0	20 (21)		
2 to <6	4 (36)	28 (29)		
6 to <18	6 (55)	37 (39)		
18 & above	1 (9)	10 (11)		
Female gender	4 (36)	32 (34)		
Residence				
India	8 (73)	84 (88)		
Bangladesh	3 (27)	11 (12)		
Symptoms at referral for sweat test				
Respiratory symptoms (persistent cough/ bronchiectasis)	11 (100)	43 (45)		
Gastrointestinal symptoms (history of meconium ileus at birth/oily stools/ chronic diarrhoea/cholelithiasis)	7 (64)	21 (22)		
Failure to thrive	6 (55)	13 (14)		
Recurrent/chronic pancreatitis	1 (9)	9 (10)		
Family history of CF	2 (18)	0		
Sweat chloride levels & sweat conductivity				
Sweat tests (mmol/l)	Quantitative sweat-chloride analysis	Sweat conductivity test	Quantitative sweat-chloride analysis	Sweat conductivity test
Mean ± SD	79.1 (17)	102.2 (14)	16.9 (9.2)	40.1 (12.2)
Median	82	99	14	38
Range (mmol/l)	61 - 106	87 - 123	7 - 45	25 - 79
Pearson correlation coefficient (r)	0.896		0.915	

CF, cystic fibrosis; SD, standard deviation

The overall accuracy of the best cut-off values was assessed through the calculation of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and κ coefficient. This showed that the best sweat conductivity cut-off value that could predict a positive CF diagnosis was ≥ 80 mmol/l with 100 per cent sensitivity, 100 per cent specificity with a PPV of 100 per cent, and an NPV of 100 per cent. Likewise, ≤ 49 mmol/l was the best sweat conductivity cut-off value that could predict a negative CF diagnosis with sensitivity of 100 per cent, specificity of 91.36 per cent, PPV of 78.13 per cent, and NPV of 100 per cent.

Intermediate range sweat conductivity values also showed a good coefficient of correlation (r_s 0.62, $P < 0.003$) with intermediate quantitative sweat chloride analysis levels (Fig. 2).

Discussion

Guidelines by the CLSI and the CFF recommend the collection of sweat following stimulation with pilocarpine iontophoresis in either gauze, filter paper or in coiled collection tubes^{9,10}. Quantitative sweat chloride estimation is the preferred diagnostic test, while the sweat conductivity test is designated as a screening test. Sweat conductivity value exceeding 50 mmol/l should be substantiated through a quantitative sweat chloride analysis^{12,13}.

Ethnic variations in sweat chloride levels have been reported by Borch *et al*¹⁴ Asian ancestry influences all three diagnostic aspects of Cystic Fibrosis. The impact is observed in the phenotype, with a higher prevalence of pancreatic sufficiency in Asian patients.

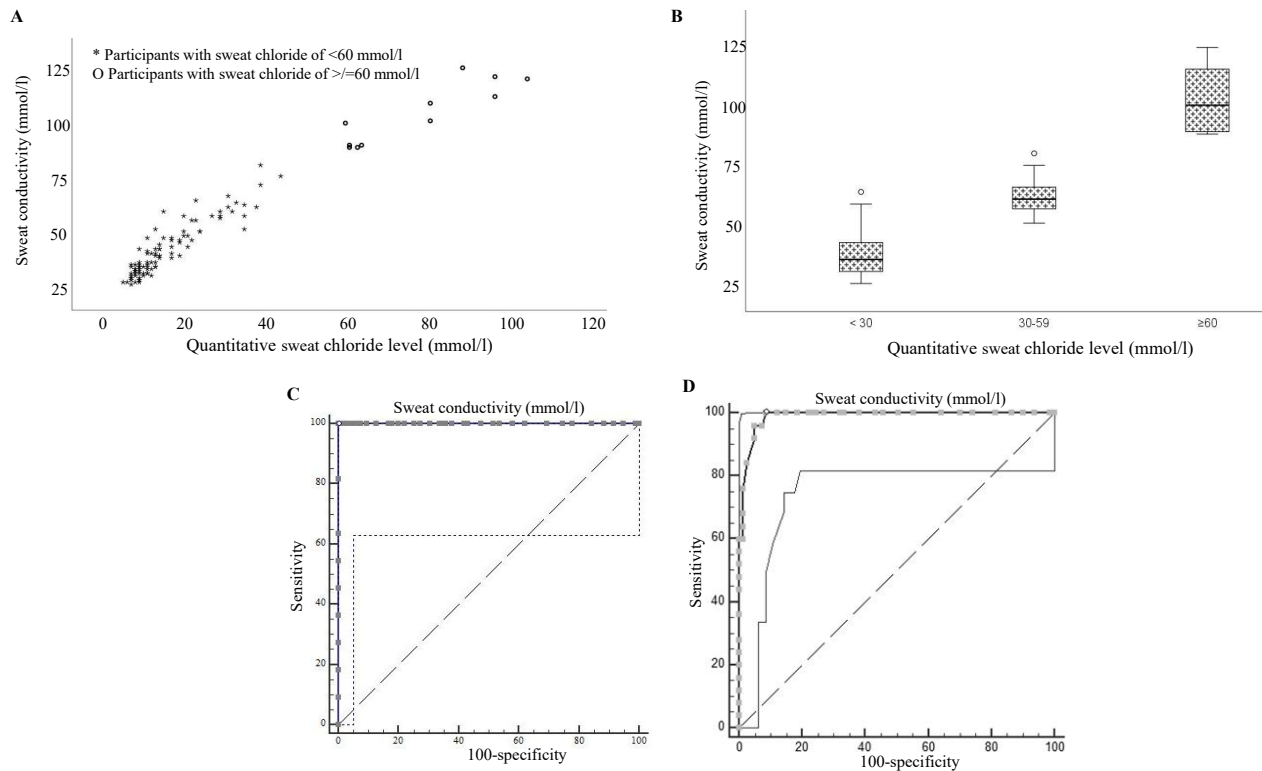


Fig. 1. The frequency distribution of sweat conductivity values plotted against sweat chloride analysis values for those with and without CF (A) revealed no overlap. (B) It illustrates that the study participants with normal and diagnostic values are clearly separated from each other. ROC curves for sweat conductivity as a diagnostic test for CF(C) shows AUC for diagnosis of CF, and (D) shows AUC for ruling out CF.

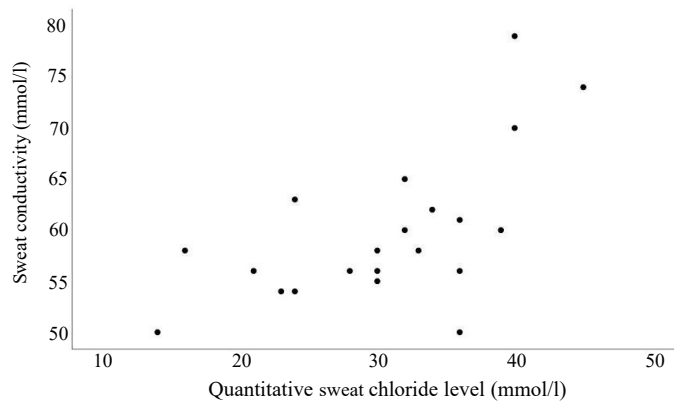


Fig. 2. Correlation of intermediate range sweat conductivity values with intermediate quantitative sweat chloride analysis levels of the cohort.

Additionally, there is a consistently lower mean sweat chloride value among individuals of Asian descent, with many values falling within the non-diagnostic range. The presence of diverse *CFTR* mutations further complicates CF diagnosis in people of Asian ethnicity, as these variations may not be effectively identified through screening panels. There are fewer studies on variability in sweat chloride values in Indian patients contributing to diagnostic dilemma^{4,15}.

Existing literature shows that sweat conductivity values agree with sweat chloride analysis in people from different ethnicities. Lezana *et al*⁸ conducted both tests on >3000 study participants in Mexico with clinical suspicion of CF and demonstrated that the sweat conductivity method using Sweat Chek analyzer (Wescor Inc) could be as reliable as quantitative sweat chloride analysis for diagnosing or ruling out CF. In parallel investigations^{9,16} in

Table II. A comparison of various available equipment for sweat testing and their benefits and challenges (conversion of INR to USD as on May 28, 2024)

Test	Equipment and processing	Expenses	Advantages	Challenges
Sweat chloride	Indigenous method (24) Induction: Pilocarpine iontophoresis Collection: Filter paper Analysis: Manual titration using Schales and Schales method	Cost of the equipment INR 2000 (24 USD)	- Inexpensive method - Has acceptable repeatability and accuracy	- 100 mg of sweat required - Errors related to technique - Room for subjective errors - Requires absolute precision of manual titration - Need for highly experienced personnel
Sweat chloride	Macroduct and chloride analyser Automated system (11,12,23) Induction: Pilocarpine iontophoresis with 0.5% pilocarpine nitrate gel disc Collection: Macroduct® Sweat Collection System (Wescor, Logan. UT) Analysis: 926 Sherwood chloride analyser	Cost of equipment Macroduct® Sweat Collection System INR 3,57,000 (4293 USD) 926 Sherwood chloride analyser INR 6,19,000 (7,443 USD)	- Only 15 µl of sweat required - Sample is analysed without dilution - Easy readout of results	- Limited availability - Expensive equipment - Maintenance of skills needed
Sweat conductivity	Macroduct and sweat check Automated system (23) Induction: pilocarpine iontophoresis with 0.5% pilocarpine nitrate gel disc Collection: Macroduct® Sweat Collection System (Wescor, Logan. UT) Analysis: 3120 SweatChek™ Sweat Analyzer (Wescor, Logan. UT)	Cost of equipment Macroduct® Sweat Collection System INR 3,57,000 (4293 USD) Sweat-Chek™ Sweat Analyzer INR 2,58,000 (3,102 USD)	- Only 15 µL of sweat required - Sample is analysed without dilution - Easy readout of results - Relatively less expensive	- Recommended only as a screening test for CF by the Cystic Fibrosis Foundation, USA
Sweat conductivity	Nanoduct (25,26) Induction (employs inbuilt Pilogel Iontophoretic Discs), collection and analysis using Nanoduct® (Wescor, Logan. UT)	Cost of the equipment INR 5,64,040 (6782 USD)	- Point of care bedside testing method - Only 3 µl of sweat required - Sensitive in neonates - Relatively less expensive	- Only few studies comparing with Sweat Chloride measurement are available

Recurring costs for each method are as follows: Indigenous method - INR 25 (0.3 USD), both sweat chloride & sweat conductivity measurement following sweat collection by macroduct system - INR 3,333 (40 US), and Nanoduct - INR 2000 (24 USD).
UT, union territory; INR, Indian rupee; USD, United States dollar

Brazil, the sweat conductivity test demonstrated an exceptional discriminatory ability in distinguishing between CF and non-CF individuals. The sweat conductivity test done in other ethnic populations in USA, Turkey, Poland, and Germany exhibited a high level of diagnostic accuracy¹⁷⁻²⁰. Bedran *et al*²¹ opined that sweat conductivity testing could be viewed as

an alternative diagnostic tool for CF in poor health infrastructure settings, given that their study revealed its excellent reliability in diagnosing CF when performed on newborns and infants younger than three months with positive newborn screening (NBS). Other authors propose that while sweat conductivity testing is reliable, positive values should undergo confirmation

through quantitative sweat chloride estimation or a genotype analysis for results in the borderline range^{22,23}.

While the *CFTR* genotype predominantly dictates sweat chloride concentration in individuals with CF, non-*CFTR* modifier genes also play a minor role in this variation²⁴. Additionally, biological factors such as age and potentially sex may influence sweat chloride levels. The influence of the above factors on sweat conductivity also needs to be explored.

Despite the demonstrated correlation with sweat chloride values, limited acceptance of sweat conductivity by international guidelines may primarily stem from its lack of specificity, as conductivity reflects a non-selective measurement of ions. It measures chloride along with other ions in the sweat, and thus conductivity values are always ~15-20 mmol/l higher than quantitative sweat Cl⁻ measurement¹⁰.

Patients with cystic fibrosis may have intermediate sweat chloride values by virtue of preserved *CFTR* function, especially when they have certain mutations. Borderline values are common in some ethnicities and young individuals. CFF recommends that these individuals undergo repeat testing at a later date and or have *CFTR* gene sequencing to identify 2 bi-allelic *CFTR* mutations for confirmation of diagnosis¹¹. A good correlation between intermediate-range sweat chloride (30 to 59 mmol/l) and sweat conductivity values (50 to 79) demonstrated in our study supports the application of the same guideline recommendation when using the sweat conductivity method.

In India, sweat testing by quantitative sweat chloride testing is not without challenges. Table II summarises the advantages and disadvantages of different equipments used for sweat testing. The pictorial representation of the equipment has been shown in the supplementary table.

The availability of sweat testing is crucial for diagnosing CF in India. As of December 2023, fewer than 10 centres in India had access to an automated chloride analyser, whereas some centres relied on indigenous method to estimate sweat chloride level (Supplementary Table). Therefore, employing sweat conductivity tests using relatively cheaper equipment can enhance the identification and timely referral of individuals suspected of having CF to specialized centres.

Our study was limited by the relatively small number of study participants; particularly those with a

definitive diagnosis of CF. There were only eight study participants below the age of one year and none in the neonatal age group. Also, our results are only applicable to sweat conductivity measurement by Sweat-Chek™ Sweat Analyzer on samples collected using the Macroduct® Sweat Collection System. Further studies are needed using Nanoduct® system (Supplementary Table) to see if similar results are obtained.

To summarise, the sweat conductivity test using Sweat-Chek™ Sweat Analyzer on sweat collected by Macroduct® Sweat Collection System showed exceptional ability to discriminate patients with CF from those without in the Indian subcontinent when quantitative sweat chloride analysis was used as the gold standard test. It also fared well to distinguish those with borderline sweat chloride level from those with normal levels.

Overall, the findings of this study suggest that in resource-poor settings cheaper sweat conductivity equipment could be reliably used wherever the sweat chloride analyser is not available. The results of this study cannot be extrapolated to values obtained *via* Nanoduct® system until further studies are done. Validation of our results by studies done on younger infants and neonates will be valuable.

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