Journal of Scientific and Innovative Research 2015; 4(2): 61-66
Available online at: www.jsirjournal.com

Research Article

ISSN 2320-4818
JSIR 2015; 4(2): 61-66

Received: 26-02-2015
Accepted: 27-03-2015

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A simple slide test for erythrocyte aggregability as a risk stratification tool in patients admitted with chest pain to the emergency room

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Abstract

Patients presenting to the Emergency Room with chest pain pose a diagnostic challenge because of a possible acute coronary syndrome. This study was done to determine the diagnostic utility of a simple slide test for red cell aggregability in patients with chest pain and as a prognostic indicator in unstable angina. 32 patients of Unstable Angina and 32 persons with non-cardiac chest pain were included in the study. A simple slide was prepared with the peripheral blood of the subjects and stained and assessed by a pathologist and by digital photographs and grid analysis to grade red cell aggregabilty. 19 patients with Unstable Angina had Grade C or D red cell aggregation while only 2 persons with noncardiac chest pain had Grade C aggregation. Estimation of Erythrocyte Percentage from the grid images and calculation of Receiver Operating Characteristic Curve gave a best cutoff value of 60-70 with a lower EP signifying unstable angina. 8 patients with Unstable Angina and an EP <50 developed acute MI or recurrent angina. To conclude, a simple slide test of the peripheral blood helps in the initial evaluation of chest pain patients for possible unstable angina, and, further has prognostic utility in patients with unstable angina.

Keywords: Slide test, Erythrocyte aggregability, Chest pain, Unstable angina.

Introduction

Acute chest pain is commonly encountered in the Emergency Room (ER) posing a diagnostic challenge to the physician who has to decide whether it is due to a life threatening pathology like acute coronary syndrome, calling for urgent management, or a more benign illness like a musculoskeletal pain or esophagitis. History, including coronary risk factors, physical examination and an electrocardiogram are most often used to differentiate a coronary event. However, the description of the chest pain alone, cannot be used to discharge a patient safely without additional testing.[1] The Electrocardiogram (ECG) too has its limitations, especially for patients with unstable angina with ischemic changes on ECG being present in only 20-30% of patients who will have a myocardial infarction (MI); so also, 5-10% of MI patients have a normal ECG on presentation.[2] Hence, rapid estimation of cardiac biomarkers, most often Troponin, has been added to the diagnostic toolkit. A quarter of patients coming to the Emergency Room with chest pain have acute MI which is quite easily diagnosed based on history, ECG and biomarkers; among the others, those at very low risk of a coronary event, based on their history and a normal ECG can be discharged and evaluated on an outpatient basis.[3] Those at intermediate risk need admission with serial ECGs and biomarkers and further investigation.[3] Various clinical scores and algorithms have been devised for chest pain evaluation but none have received universal acceptance.

A simplistic explanation of the pathophysiology of acute coronary syndrome postulates that disruption of the atherosclerotic plaque or endothelial erosion exposes the lipid core or subendothelial connective tissue respectively, which causes platelet aggregation and subsequently fibrin deposition.[4] Inflammation has also been shown to play an important role in the pathogenesis of acute coronary syndrome and the inflammation in an atherosclerotic plaque has been compared to be of equal intensity as that in the synovium in acute rheumatoid arthritis.[4] Platelet activation has been shown to occur in acute MI and persist even four weeks later, though attenuated by treatment[5]; further, spontaneous platelet aggregates have been demonstrated with a laser light aggregometer in patients with acute MI.[6] Sticky platelets have also been found to aggregate with leukocytes with platelet molecules like P-selectin, ICAM-2 and Glycoproteins interacting with neutrophil PSGL-1, LFA-1 and Mac-1.[7]

Similarly, erythrocyte aggregation has also been demonstrated in patients with unstable and stable coronary artery disease.[8, 9] We had earlier shown that increased erythrocyte aggregability can be demonstrated by a simple slide test in patients with acute MI and stroke.[10] Guidelines recommend a quick assessment of patients presenting with chest pain, including history and examination, an ECG, and 1 or 2 serial measurements of cardiac enzymes.[11] The slide test for red cell aggregability is a simple and inexpensive test that can be performed anywhere and would be a valuable diagnostic tool if it is shown to be have significant diagnostic utilty. However the available data are not robust enough to make definite recommendations in guidelines. Further while some studies have found the slide test to have some prognostication value in patients with unstable angina[12, 13], at least one study has found that the erythrocyte aggregation is increased in both unstable an stable coronary artery disease and that it is unlikely to contribute to emergence of acute myocardial infarction.[8] Against this background, this case – control study was planned to determine the feasibility of using a simple inexpensive slide test for erythrocyte aggregability as a diagnostic tool to triage patients with chest pain in the ICCU.

Aim of the study

To study the diagnostic utility of a simple slide test for erythrocyte aggregability in patients with unstable angina vs. patients with noncardiac chest pain

To study the utility of the simple slide test as a prognostic indicator in patients with unstable angina

Methodology

32 patients of Unstable Angina (UA) were included in the study. UA was diagnosed on the basis of chest pain for at least 10 minutes, rest angina or recent onset angina (within the past one month) or accelerating angina, and, ECG evidence of ST segment depression or T inversion, and no significant elevation of enzymes. Unstable angina was classified as per Braunwald and Hamm classification as follows[14] –

I – New onset of severe angina or accelerating angina; No rest pain

II – Angina at rest in the past one month, but Not in the preceding 48 hours

III – Angina at rest within 48 hours

The patients of UA were further subdivided into those with ECG changes and those without.

Exclusion criteria were (a) presence of ST elevation MI or non-ST elevation MI on admission, as evidenced by, ECG evidence of ST elevation (STEMI) or a twofold or greater rise in cardiac enzymes and (b) administration of any antithrombotic / anticoagulant treatment prior to admission.

32 patients admitted to the ER for non-cardiac causes like musculoskeletal pain, asthma, reflux esophagitis, etc., served as controls.

Approval from the Institutional Ethics Committee was obtained prior to conduct of the study. Informed consent was taken from the patients. All patients were subjected to a history and physical examination, routine blood counts and chemistry, ECG, CPK-MB & Troponin T and Echocardiography. Further investigations were decided upon, by the treating physician.

Slide test for erythrocyte aggregability

This was described previously by us.[10] A blood sample was collected immediately upon admission and before any treatment was started. A drop of citrated blood (one volume of citrate to three volumes of blood) was put on a glass slide positioned at a slope of 45 degrees and allowed to run down by gravity leaving a fine film. The slide was then stained with Leishmann stain.[10]

The slide was subjectively assessed by the pathologist who was blinded to the diagnosis. The slide was studied at 200x magnification and a grade was assigned to the slide based on the degree of erythrocyte aggregation (Figure 1) as follows:

Grade A: Erythrocytes are discrete with uniform distribution throughout the slide and not aggregated, clear areas not seen (Normal aggregation).

Grade B: Erythrocyte aggregates are seen in some areas of slide with small clear spaces (Mild aggregation).

Grade C: Variable sizes of aggregates over all areas of slide with small clear spaces (Moderate aggregation).

Grade D: Large thick aggregates with rounded/ clear borders and large clear spaces (Severe aggregation).

Next, digital images were randomly taken from 9 areas - 3 from the head of the smear, 3 from the body and 3 from the tail of the smear. In each image, the degree of aggregation was quantified using a variable called erythrocyte percentage (EP) which is defined as percentage of image area occupied by erythrocytes. If there is more aggregation there will be more clear

spaces and a reduction in the area occupied by erythrocyte – that is, the EP is decreased.

To measure the erythrocyte percentage we used a grid available in Abode Photoshop where the average number of area occupied by RBC was calculated for the 9 grid images taken from each slide.

EP (Erythrocyte Percentage) = (Average number of squares occupied by RBC) / (Total number of squares) x 100.

Grading of smears by the pathologist from microscopy, and, calculation of EP from the grid images, was done for all the smears taken from patients with unstable angina and persons with chest pain due to other causes.

All patients were followed upto one week for any development of acute myocardial infarction (as evidenced by ST elevation or more than twice rise in cardiac enzymes) or complications like acute left ventricular failure, hypotension, recurrent angina, need for urgent revascularization or death.

All data were tabulated and results of both groups compared using chi square test to compare proportions. A p value of <0.05 was considered as statistically significant.

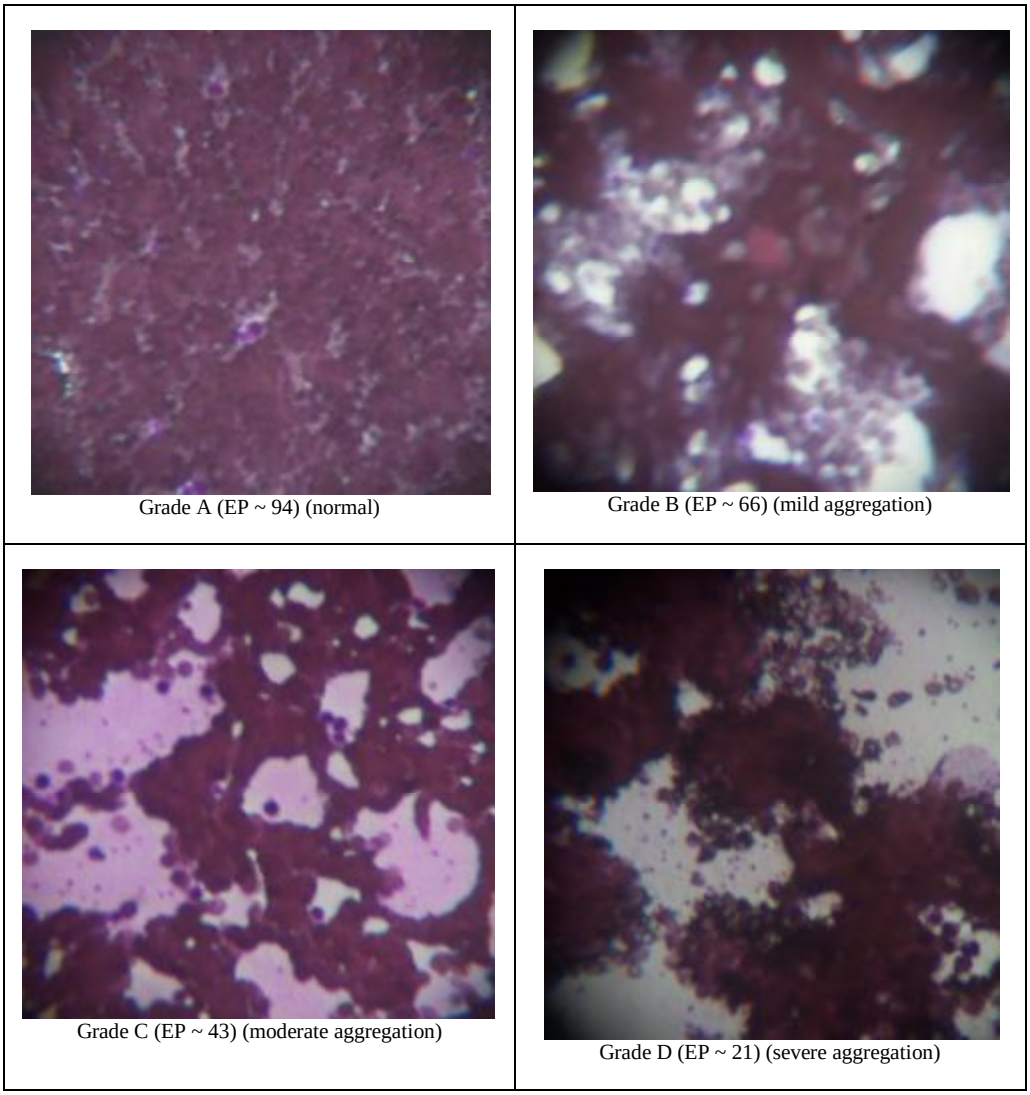

Figure 1: Degree of erythrocyte aggregation

Results

The demographic profile is given in Table 1. Both groups showed a male preponderance.

Table 1: Demographic profile of patients in the Unstable Angina group and Non-cardiac chest pain group

	Unstable angina group	Non-cardiac chest pain group	Remarks
Total no. (n)	32	32	
M:F	19 : 13 (1.46:1)	18 : 14 (1.28:1)	
Mean age (SD) years	57 (11.6)	40.9 (12.2)	
Hypertension	23		
Diabetes	21		
Smoking	4		

The grades of erythrocyte aggregation in both the groups are shown in Table 2. There was a significant difference between the two groups – 19 patients of unstable angina had Grade C or D red blood cell (RBC) aggregation while only 2 patients with nonischemic pain had Grade C aggregation (p=0.031). Using Grade C or D (vs. Grade A or B) to diagnose unstable angina gave a sensitivity of 59% and a specificity of 93%. Using B or C or D grades (vs. Grade A) to diagnose unstable angina gave a sensitivity of 87% and a specificity of 68%. Degree of erythrocyte aggregation increases from grade A to grade D.

Table 2: Grade of erythrocyte aggregation among patients with unstable angina and non-cardiac chest pain

Grade of Erythrocyte aggregation on peripheral smear	Unstable angina (n=32)	Non cardiac chest pain (n=32)
A	4	22
B	9	8
C	15	2
D	4	0

Estimation of the 'Erythrocyte Percentage' (EP - which value is inversely proportional to erythrocyte aggregation) from the grid images, showed that in none of the patients with noncardiac chest pain was the EP less than 50, while 24/32 patients (75%) with UA had an EP below 60, indicating moderate to severe red cell aggregation (Figure 2).

EP is the area occupied by erythrocytes expressed as a percentage of total image /slide area. EP is inversely proportional to degree of erythrocyte aggregation.

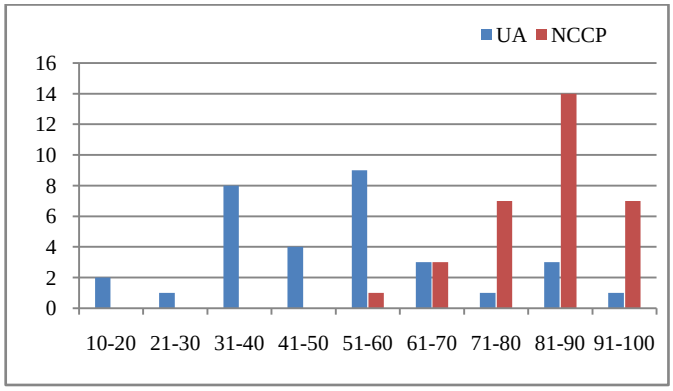

Figure 2: Erythrocyte Percentage (EP) in patients with Unstable Angina and Noncardiac Chest Pain.

A Receiver Operating Characteristic Curve (ROC curve) using the Erythrocyte Percentage gave the best cutoff point between 60 and 70 with a lower EP signifying unstable angina (Figure 3).

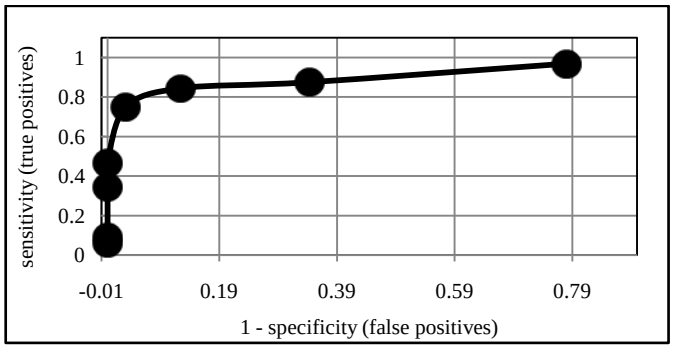

Figure 3: ROC curve (Receiver Operating Characteristic Curve) of Erythrocyte Percentage cutoff value from 20% to 90%.

Furthermore, of the 3 patients with UA who had an EP <30, one developed myocardial infarction and one had recurrent angina during hospital stay. Of the 12 patients of UA with an EP 31-50, 1 developed MI and 5 patients had recurrent angina. None of the 17 patients of UA with an EP >50 had recurrent angina. (Table 3)

Table 3: 1 week outcome of Unstable Angina patients in relation to Erythrocyte Percentage. (EP is inversely proportional to Erythrocyte aggregation)

Erythrocyte Percentage (EP)	No. of patients with UA	No. who had recurrent angina / acute MI during 1 week of hospital stay
< 30	3	2 (66.7%)
31-50	12	6 (50%)
> 50	17	0

When patients of UA were studied for the relation between class of unstable angina and subjective grading of erythrocyte aggregation, it was found that there were more patients of UA Class III who had Grade C or D erythrocyte aggregation (Table 4).

Table 4: Grading of erythrocyte aggregation in the peripheral smear in the different groups of UA patients

Grade of RBC aggregation	UA Class II without ECG changes	UA Class II with ECG changes	UA Class III without ECG changes	UA Class III with ECG changes
A	2	2	0	0
B	2	3	2	2
C	2	1	5	4
D	0	0	4	3

Re-grouping of the patients into two groups based on UA class (II vs. III) and subjective grading of RBC aggregation (A+B vs. C+D) (Table 5) showed that, 9 of 12 patients with UA Class II had Grade A or B aggregation while 16 of 20 patients with UA Class II had Grade C or D aggregation (p=0.002).

Table 5: Relation between UA class and grade of erythrocyte aggregation in the peripheral smear (p=0.002)

	Unstable Angina Class II	Unstable Angina Class III
RBC aggregation Grade A & B	9	4
RBC aggregation Grade C & D	3	16

Similarly there was a significant association between grading of RBC aggregation vs. development of recurrent angina in the patients admitted for UA (Table 6). 14 patients of UA with Grade A or B erythrocyte aggregation had No recurrent angina, while, 44,4% (8/18) patients of UA who had Grade C or D erythrocyte aggregation had recurrent angina.

Table 6: Relation between degree of erythrocyte aggregation and development of recurreng angina (p=0.007)

	No pain in hospital	Recurrent angina
Grade A or B erythrocyte aggregation (no or mild aggregation)	14	0
Grade C or D erythrocyte aggregation (moderate or severe aggregation)	10	8

Discussion

The present study was aimed at determining the utility of a simple slide test for erythrocyte aggregation in patients presenting to the ER or ICCU with chest pain. Determining the cause of chest pain in patients can be challenging and the problem is accentuated by the limitations of existing diagnostic tests for acute coronary ischemia.[15] Fearing the consequences of missing an acute coronary syndrome, emergency doctors tend admit even doubtful cases and upto 60% of cases admitted to the ER eventually are found to have noncardiac pain.[15] In a study, the initial ECG had a sensitivity of only 55% for acute MIs and 27% for unstable angina.[16]

In the present study, 22/32 (68.75%) patients with noncardiac pain had no red cell aggregation in the peripheral smear (Grade A) while only 2 of them (6.25%) had Grade C aggregation with none having Grade D. On the other hand, among the patients with Unstable Angina, 15+4 patients had Grade C and D aggregation respectively (19/32 = 59.38%), which difference was statistically significant. Division of the data into a binary model (Grade C+D vs. Grade A+B) gave a sensitivity of 59% and specificity of 93% to diagnose unstable angina. Using a binary model of Grade B+C+D vs. Grade A alone (any aggregation vs. no

aggregation) improved the sensitivity to 87% but specificity decreased to 68%.

Similarly, when the erythrocyte percentage was estimated and an ROC curve derived, the best cutoff point was 60-70 with a lower value indicating unstable angina. Earlier studies have demonstrated increased red cell aggregation in acute coronary syndrome but the present study has shown that moderate to severe aggregation of erythrocytes (Grade C & D) on a simple slide test and estimation of Erythrocyte Percentage are two bedside tests that have an acceptable sensitivity and specificity in the diagnosis of unstable angina and noncardiac chest pain.

Several studies have shown increased red cell aggregation in acute MI[10, 17] and unstable angina; prognostic utility too has been demonstrated in some studies in UA[12, 13] and MI[18] but others have not found any relation between erythrocyte aggregation and adverse events in acute MI[8, 12]. However, an in vitro study with blood samples from patients with acute MI and UA found that red cell aggregation tends to occur in conditions of slow flow only and concludes that such events can adversely affect the microcirculatory flow in patients with acute coronary syndromes[19] – the authors postulate the use of low dose thrombolysis after angioplasty. Another study of erythrocyte aggregation among patients with acute coronary syndrome has reported that erythrocyte aggregation and inflammatory biomarkers increase as time from symptom onset lengthens in patients with acute MI[20] suggesting that the pathophysiological process persists for some time.

The present study found an association between increased erythrocyte aggregability (subjective grading as well as by the grid method) and severity of unstable angina (Table 4 & 5). Similarly, 2 of the 3 patients of UA who had an Erythrocyte Percentage (EP) < 30 (indicating increased red cell aggregation) had adverse outcomes during hospital stay with 1 MI and one recurrent angina (Table 3) while half of the 12 patients had such adverse outcomes in the hospital (1 MI and 5 recurrent angina) while none of those with an EP >50 had such outcomes. These findings support the hypothesis that increased red cell aggregation leads to an increased risk of adverse events in patients with unstable angina.

A similar study in 89 patients with proven ischemic heart disease and who were admitted with chest pain but had no MI on admission showed that enhanced erythrocyte aggregation / adhesiveness was associated with an eventful course (worsening of symptoms, hospitalization for unstable angina / MI or heart failure or angioplasty / CABG over the next 6-12 months.[21]

Platelet aggregation is a key event in the initiation of an acute coronary syndrome. Beginning with von Willebrand factor facilitated platelet adhesion to collagen and tissue factors exposed by rupture of an atherosclerotic plaque, and, platelet activation releasing thromboxane A2, serotonin and other platelet aggregatory substances, there is activation of the GpIIb/IIIa receptors facilitating formation of platelet plug and fibrin deposition and inclusion of erythrocytes in the clot.[22, 23] Using a laser based aggregometer, the peripheral blood of all of 39 patients with acute MI /UA but none of 14 healthy controls were found to have primary platelet aggregates (small, < 100 platelets)[6]– thus circulating platelets which, in healthy individuals are not expected to aggregate, were shown to form primary aggregates at an enhanced rate in patients with acute coronary syndrome. Such tendency to aggregate has also been reported in erythrocytes mediated by fibrinogen[19] and in leukocytes[24]. A study of the thrombi aspirated from patients of acute MI during primary angioplasty has shown that patients with high red cell thrombi were more likely to have impaired myocardial perfusion, as evidenced by incomplete ST segment resolution on ECG, lower myocardial blush grades and progression of adverse LV remodeling upto 6 months.[19] Release of myeloperoxidase from activated neutrophils and monocytes and oxidized LDL may increase the aggregabiity of erythrocytes and their adhesiveness to the endothelium.

As currently available diagnostic modalities are not fully accurate or reliable in the evaluation of patients with chest pain in the ICCU, several other tests have been studied with some utility. Of these, the Coronary Calcium Score has a good negative predictive value but poor positive predictive value.[25] CT Coronary Angiography also has a good sensitivity and negative predictive value[25] but carries the problem of accessibility, and, problems of contrast allergy and renal failure. Noninvasive tests like stress echocardiography and myocardial perfusion scintigraphy need specialized equipment but do not seem to alter the outcome / risk among patients with chest pain who are diagnosed to be at low risk for an acute coronary event and can be deferred.[26]

The present study has found the slide test for erythrocyte aggregation to be a simple test of reasonable sensitivity and specificity in patients presenting with chest pain and also to be of prognostic utility in patients with unstable angina. This test was found to be a useful 'biomarker' for detection of low grade inflammation in men with atherothrombotic vascular disease – the receiver operating characteristic curve analysis found it to be superior to other commonly used markers.[27] A study in healthy individuals found this simple slide test for red cell aggregability to correlate significantly (r=0.5-0.6) with high sensitive C Reactive Protein levels, fibrinogen levels and erythrocyte sedimentation rate.[28] Commercial tests for erythrocyte adhesiveness like Inflamet are available[29] but can be expensive and may not be available at all centres. However, the present study has shown that the simple slide test can also be graded subjectively or the Erythrocyte Percentage (EP) measured objectively using digital images with a grid method, and, that this test is reasonably helpful (sensitivity of 59-87% and specificity of 68-93%) in the initial evaluation of chest pain to detect unstable angina and also as a risk stratification tool.

Conclusion

A simple slide test of peripheral blood can be effectively used to detect and grade erythrocyte aggregability and can be used in the Emergency Room to evaluate patients with acute chest pain for any evidence of unstable angina which is associated with increased red cell adhesiveness. The test also carries prognostic utility in patients with unstable angina.

Acknowledgements

The present study was done by Ram Manohar Talupula as part of the ICMR sponsored STS under the guidance of Prasad Uma.

Conflict of interest: None.

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