First Indian initiative for preparation of low-titer group “O” single-donor platelets with platelet additive solution

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Abstract:
BACKGROUND: Guidelines recommend ABO-identical platelet (PLT) transfusions. Hemolytic reactions after a minor ABO-incompatible PLT transfusion have escalated due to single-donor platelets (SDP) containing ABO-incompatible plasma. Avoiding such events by examining titers or performing plasma reduction is cumbersome. The introduction of platelet additive solutions (PAS) has enabled to reduce these reactions by avoiding passive transfer of isoagglutinin. Our aim was to study antibody titers (anti-A, anti-B) in “O” SDP by adding PAS at source and the quality parameters with reference to viability, morphology, and metabolism.

MATERIALS AND METHODS: Group “O” SDP (n = 50) were prepared on a standard cell separator. PAS in a ratio of 70:30 (PAS: plasma) was added at source under sterile conditions (study arm). The units were studied on day of collection (day 0) and day 4 and compared with SDP containing 100% plasma (control arm). A titer study was performed after PAS addition.

RESULTS: In the study group, the median antibody titers (anti-A, anti-B) reduced from 128 to 16, post-PAS addition (P < 0.001). Morphology scores were superior in PAS platelet concentrates (P < 0.001). Metabolic parameters PO2 and PCO2 were similar in the two arms signifying good unit storage and stable oxygen consumption (P > 0.05). Lactate levels, glucose consumption rate, and lactate production rates were significantly low in study arm showing the advantage of PAS.

CONCLUSION: O group SDPs can be prepared with PAS and the beneficial effects were significant with respect to antibody titers. Quality parameters were well maintained. Availability of PAS units has benefitted patients.

Keywords: Group O platelets, low titer, platelet additive solutions, quality parameters, single-donor platelets

Introduction

Platelet concentrates (PC) are a scarce resource in hospitals dealing with a large number of oncology patients. They form an important component of supportive care in these patients, thus making maintenance of platelet (PLT) inventory a challenging task. International guidelines recommend transfusion of group-specific PC to yield good increments in thrombocytopenic patients. However, group switchover is an acceptable practice when group-specific PCs are not available. Major ABO-incompatible PLT transfusions (transfusion of PLTs carrying antigens to which recipient has naturally occurring antibodies) are associated with poor increments due to accelerated destruction of the transfused PLTs in recipients. Minor ABO-incompatible PLT transfusions (transfusion of PLTs carrying naturally occurring antibodies against antigens on recipient red cells) are associated with incidences of hemolysis of recipient red cells which at times can be fatal. Different blood transfusion services have devised their own policies on the issue of group switchover with respect to PLT transfusion. With increasing

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use of single-donor platelets (SDP), there have been adverse events related to minor ABO-incompatible PLT transfusions in patients. Approximately forty cases have been reported in the literature which highlights the problems of passive transfer of antibodies in such events. The problem is compounded while using group “O” SDP as these donors may have high titers of naturally occurring anti-A and anti-B antibodies. Various approaches have been suggested to reduce the hazards of minor ABO-incompatible PLT transfusions. These are studying titer levels in donors and defining safe titer levels, reducing the plasma volume of the intended unit, washing the PC with saline before issue, and replacing incompatible plasma with either AB plasma or the use of platelet additive solution (PAS).

Issuing group O units after studying their titer levels is not followed universally, due to lack of consensus over the safe titer levels and differences in methodology adopted to perform titer level studies. PC manipulation by reducing the volume of incompatible plasma from the unit or washing with saline requires technical expertise, is time-consuming, and additional labor costs are involved. It has been reported that concentrating or washing PLTs lead to reduction in number of PLTs transfused and substandard corrected count increment in patients. PAS being an isotonic buffered solution acts as a substitute for a substantial fraction of plasma in a PC. PLTs can be stored with different PAS solutions by replacing 65%–80% of plasma, thus reducing plasma proteins, including isoagglutinin. Apart from reducing the risk of hemolysis due to passive transfer of naturally occurring antibodies, the use of PAS has led to a reduction in number of allergic transfusion reactions and the extra-recovered plasma may be sent for fractionation.

PAS IIIM (SSP+, Macopharma, Mouvaux, France) has been recently approved by Drug Controller General of India for commercial use in our country. At present, there are no published reports on the effect of PAS on quality parameters and in vitro variables of SDP from India. This study was conducted to explore the feasibility of using PAS for making low-titer group “O” units which can be transfused across the ABO barrier and also maintain acceptable standards for quality parameters over the duration of storage (5 days) when compared with PC produced with 100% plasma.

Materials and Methods

The project was approved by the Institutional Review Board and partly funded intramurally. PAS was procured from Macopharma (France) which supported the project. The study arm comprised group “O” SDPs suspended in 70% PAS and 30% plasma and the control arm comprised A, B, or O group SDPs suspended in 100% autologous plasma.

Platelet collection, preparation, and storage

Fifty PC for each arm were collected on cell separator (version 3.2, Amicus®, FreseniusKabi, Germany), from eligible PLT donors after an informed consent. The PCs for study arm were collected from group O donors only while those for the control arm were collected from donors of any ABO blood type on the designated cell separator. The device has an option of selecting a procedure with either 100% plasma or with 70% PAS, at the beginning of kit installation. The addition of PAS into the SDP was performed during resuspension of PLT pellet at the end of PLT collection. A nonpaired design was adopted to reflect the standard method of processing. PAS (SSP+™, MacoPharma, Moveaux, France), licensed in India, was used for the study arm. The final volume and yield of PC were set at 300 ml and 4 × 10^11 PLTs/unit, respectively. The PCs were left to rest for 1 h at room temperature and then transferred for storage in a PLT incubator agitator (Model 3603/4720, Forma, Thermo Electron Corp., Ohio, USA) with a stable temperature of 22°C ± 2°C.

Sampling

Units were stored for 5 days, and PLT samples obtained aseptically from these units 6 h after collection (day 0) and on day 4. Representative samples were obtained by gentle mixing and segment stripping of the PLT unit. At each time, a 5–8 ml sample was collected in a sample pouch connected to the PLT storage container. For performing sterility checks and morphology score, a segment sample attached to the PLT storage container was used.

Isoagglutinin titer levels in study arm single-donor platelets

Titers were tested using doubling dilution technique. Serial two-fold master dilutions of sample (donor plasma from pilot tube and PC from sample pouch) were prepared in 0.9% saline. Samples were tested for anti-A and anti-B by direct agglutination using the tube technique. The antibody titer in the current study was defined as the reciprocal of the highest dilution of plasma that produced visible agglutination. The titer of anti-A and anti-B from donor pilot tube sample was reported as pre-preparation titer and corresponding sample from the study arm unit (group “O” SDP in PAS) as the post-preparation titer.

Platelet concentration, mean platelet volume, platelet yield, swirling

PLT concentration and mean platelet volume (MPV) were measured on a hematology analyzer (Sysmex KX-21, Sysmex Corp., Japan). The PLT yield was
calculated using PLT concentration and unit volume. Swirling was assessed by visual inspection and graded as 0 (no swirling), +, ++, or +++ (maximum swirling).\textsuperscript{[13]}

Leukodepletion, morphology score (Kunicki score)
All samples were assessed for leukodepletion manually by Nageotte’s chamber. Morphology score for PLTs as described by Kunicki et al. was performed using a phase-contrast microscope.\textsuperscript{[14]}

Confirmation of sterility
Sterility tests were performed on day of collection at 6 h after collection, using the BacT/ALERT system for aerobic and anaerobic cultures (Biomerieux, France). Volume of inoculation was 5 ml.

Blood gases and metabolic variables
pH, blood gases (pO\(_2\), and pCO\(_2\)), and metabolic variables (glucose, lactate, and bicarbonate) were measured at 37°C using a blood gas analyzer (Cobas221b, Roche Diagnostics, Inc.) immediately after sampling. Glucose consumption rate (GCR) and lactate production rate (LPR) in (mmol/day/10\(^{12}\) PLTs) were calculated.

Statistical analysis
Results are presented as mean ± standard deviation. PLTs in both arms were analyzed using repeated-measures ANOVA. Samples were analyzed on day of collection (day 0) and on day 4 using two-tailed “paired” t-test. A P < 0.05 was considered statistically significant for all tests. All data were analyzed using computer software IBM\textsuperscript{®} SPSS\textsuperscript{®} Statistics, Version 22 (IBM Corp. Released 2013. Armonk, NY, USA).

Results

Anti-A and anti-B titer levels in study arm platelets
The median titer of anti-A and anti-B for all donor plasma samples was 128. The median titer of anti-A and anti-B from the PAS PC sample was 16. No study arm units had anti-A and anti-B titers more than 64. Geometric mean titer and range are represented in Table 1. Fifty-six percent (28/50) of donor samples showed titers of anti-A and anti-B ≥128 [Table 2]. The reduction in titer of anti-A and anti-B after addition of PAS is represented in Tables 3 and 4, respectively, and was statistically significant (P < 0.05).

Swirling, leukodepletion, and bacterial contamination
Swirling gradation by visual inspection was maximum (Grades 0 to ++++) in all PCs (control and study arm) on day 0 and day 4. Leukodepletion as defined by white blood cell count <1 × 10\(^{9}\)/unit was achieved in all PCs, when tested on day 0. Bacterial contamination was not reported in any of the PCs on day 0.

Platelet quality parameters
PC volume, PC yields, and concentration were similar in study and control arm on day 0 and day 4. There was no significant reduction in the volume, yield, or concentration in either arm (paired t-test) by day 4. MPV was similar in both arms on days of testing. When

\[\text{Table 1: Pre- (donor) and post- (platelet additive solution added unit) titers of study arm}^* (n=50)\]

| Isoagglutinin | Pre-titers | Post-titers |
|---------------|------------|-------------|
| Anti-A        | 160.1±136.2 (32-512) | 22.2±18.34 (4-64)³ |
| Anti-B        | 156.7±141.5 (4-512)  | 22.1±20.25 (N-64) ³ |

*Data are reported as geometric mean±SD (range), \(1^{P}<0.001\) versus pre-procedure titers. SD = Standard deviation, N = Not detectable

\[\text{Table 2: Study arm platelets with titers} \geq 128^* (n=28/50)\]

| Isoagglutinin | Pre-titers | Post-titers |
|---------------|------------|-------------|
| Anti-A        | 246.5±127.6 (128-512) | 33.8±17.93 (16-64)³ |
| Anti-B        | 256±122.9 (128-512)  | 36.1±20.25 (16-64)³ |

*Data are reported as geometric mean±SD (range), \(1^{P}<0.001\) versus pre-titers. SD = Standard deviation

\[\text{Table 3: Extent of titer reduction (anti-A) after platelet additive solution addition in study arm platelet concentrates}\]

| Anti-A pre-procedure titer | Anti-A post-procedure titer | Total |
|---------------------------|----------------------------|-------|
| N                         | 2                          | 4     | 8     | 16   | 32   | 64   |
| 0                         |                            |       |       |      |      |      |
| 2                         |                            | 0     |      |      |      |      |
| 4                         |                            | 0     |      |      |      |      |
| 8                         |                            | 0     |      |      |      |      |
| 16                        |                            | 0     |      |      |      |      |
| 32                        |                            | 5     | 3    | 1    | 9    |
| 64                        |                            | 9     | 4    | 13   |
| 128                       |                            | 9     | 1    | 10   |
| 256                       |                            | 12    | 2    | 14   |
| 512                       |                            | 4     | 4    |      |
| Total                     |                            | 0     | 5    | 12   | 14   | 13   | 6    | 50^* |

*Numbers in result columns indicate number of PLT units. N = Not detectable, PLT = Platelet

\[\text{Table 4: Extent of titer reduction (anti-B) after platelet additive solution addition in study arm platelet concentrates}\]

| Anti-B pre-procedure titer | Anti-B post-t procedure titer | Total |
|---------------------------|-------------------------------|-------|
| N                         | 2                             | 4     | 8     | 16   | 32   | 64   |
| 0                         |                               |       |       |      |      |      |
| 2                         |                               | 0     |      |      |      |      |
| 4                         |                               | 0     |      |      |      |      |
| 8                         |                               | 0     |      |      |      |      |
| 16                        |                               | 0     |      |      |      |      |
| 32                        |                               | 8     | 1    | 10   |
| 64                        |                               | 9     |      |      |
| 128                       |                               | 7     | 1    | 8    |
| 256                       |                               | 12    | 3    | 15   |
| 512                       |                               | 4     | 4    |      |
| Total                     |                               | 1     | 11   | 10   | 8    | 13   | 7    | 50^* |

*Numbers in result columns indicate number of PLT units. N = Not detectable, PLT = Platelet
individual arm was assessed, a significant increase in MPV was found over storage period of 5 days [Table 5]. Morphology score by Kunicki method was studied in both arms and found to be superior in the study arm on day 4 [Table 5].

Blood gases ($pO_2$, $pCO_2$, pH, and metabolic parameters (glucose, lactate, bicarbonate)

$pH$ was similar in study and control arm PCs on day 0 but on day 4, PAS PCs had a lower $pH$ which was within acceptable limits ($P < 0.05$) [Table 6].$^1$ $pO_2$ and $pCO_2$ were similar in study and control arms on day 0 and day 4. $pO_2$ increased and $pCO_2$ decreased during storage for all PCs and the storage-induced change was similar for both the arms. Metabolic variables are shown in Table 6. Glucose levels on day 0 were lower in study arm as compared to control arm ($P < 0.05$). Lactate levels were similar in the two arms on day 0. Glucose decreased and lactate increased ($P < 0.05$) from day 0 to day 4 in PC from both arms. The control arm PC metabolized glucose and produced lactate at a significantly higher rate compared to the study arm PC [Table 7]. There was a statistically significant difference in the storage-induced change in glucose and lactate between control and study arms.

| Characteristic                  | Arm   | Day          | Day          |
|--------------------------------|-------|--------------|--------------|
| PLT volume (mL)                | Control | 293.98±7.23 (276-320) | 282.62±7.19 (265-310) |
|                                | Study  | 286.3±6.82 (265-299) | 272.8±7.57 (254-288) |
| PLT yield ($10^{11}$/unit)     | Control | 4.29±0.84 (2.51-6.27) | 4.10±0.72 (2.43-5.49) |
|                                | Study  | 4.52±0.75 (2.71-6.36) | 4.32±0.67 (2.74-5.90) |
| PLT concentration ($10^9$/L)   | Control | 1459.6±278.9 (871-2150) | 1448.9±253.5 (870-1899) |
|                                | Study  | 1576.85±262.1 (931-2150) | 1569.68±249.8 (987-2150) |
| MPV (fL)                       | Control | 7.9±1.31 (5.6-12.5) | 8.25±1.39 (5.9-12.7)$^a$
|                                | Study  | 7.68±1.14 (6.1-10.8) | 8.04±1.15 (6.3-11.1)$^a$
| Morphology score (/400)        | Control | 310±19.39 (260-354) | 265±36.1 (188-310)$^a$
|                                | Study  | 320±18.66 (250-346) | 285±21.8 (211-328)$^a$ |

*Data are reported as mean±SD (range); $n$=50, $^a$Repeated-measures ANOVA ($P<0.0045$ versus control), $^b$Paired $t$-test ($P<0.05$ versus day 0). PLT = Platelet, MPV = Mean platelet volume, SD = Standard deviation

| Characteristic                  | Arm   | Day          | Day          |
|--------------------------------|-------|--------------|--------------|
| pH at 37°C                      | Control | 7.11±0.09 (6.88-7.31) | 6.99±0.27 (6.3-7.03)$^b$
|                                | Study  | 6.99±0.13 (6.64-7.17) | 6.91±0.10 (6.57-7.13)$^b$
| $pO_2$ (kPa)                    | Control | 14.12±1.60 (10.35-17.22) | 15.25±2.04 (11.8-20.88)$^b$
|                                | Test   | 14.86±1.56 (11.64-18.15) | 15.71±1.88 (11.81-18.80)$^b$
| $pCO_2$ (kPa)                   | Control | 5.34±1.03 (3.03-7.97) | 3.23±0.68 (1.25-4.55)$^b$
|                                | Test   | 2.59±0.56 (1.42-3.87) | 2.08±0.39 (1.38-2.89)$^b$
| Glucose (mmol/L)                | Control | 15.30±1.86 (10.27-18.20) | 9.90±2.75 (2.55-14.93)$^b$
|                                | Study  | 4.84±1.30 (1.89-7.38)$^c$ | 2.11±0.83 (0.94-4.16)$^c$ |
| Lactate (mmol/L)                | Control | 5.25±2.65 (2.10-13.20) | 14.17±3.71 (7.10-21.50)$^c$ |
|                                | Test   | 5.89±2.77 (1.50-14.10) | 11.89±2.29 (7.20-17.20)$^c$ |
| Bicarbonate (mmol/L)            | Control | 17.50±2.37 (10.9-21.8) | 8.75±3.01 (5.10-13.10)$^c$ |
|                                | Test   | 6.30±1.07 (3.80-8.50)$^c$ | 4.34±1.08 (2.60-7.40)$^c$ |

*Data are reported as mean±SD (range); $n$=50, $^a$Repeated-measures ANOVA ($P<0.0045$ versus control), $^b$Paired $t$-test ($P<0.05$ versus day 0). SD = Standard deviation

| Variable                       | Control arm | Study arm | $P$    |
|--------------------------------|-------------|-----------|--------|
| Lactate production rate        | 0.53±0.17 (0.05-0.89)$^a$ | 0.34±0.14 (0.02-0.64) | $<0.01$ |
| Glucose consumption rate       | 0.317±0.13 (0.11-0.64) | 0.157±0.07 (0.01-0.30) | $<0.01$ |
| Ratio                          | 1.83±0.59 (0.18-3.35) | 2.25±1.23 (0.36-7.03) | 0.032  |

*Data are reported as mean±SD (range); $n$=50. SD = Standard deviation
**Discussion**

In principal, ABO identical PC are recommended for optimal increments in PLT counts in patients.[2] However, practically, this is not always feasible due to scarcity of group-specific PLTs, especially SDP’s, in times of urgent need. This is compounded by the fact that PC has a short shelf life of 5 days, wherein strict adherence to the policy of transfusing ABO identical PLTs can lead to outdate and expiry of PC.[16] In patients with refractory thrombocytopenia, it is difficult to find human leukocyte antigen (HLA) compatible donors who are also ABO identical. In such cases, it has been reported that HLA-matched and minor ABO-incompatible PLTs are more effective than ABO-compatible but only partially HLA-compatible units.[7] Thus, it is important to make minor ABO-incompatible PLT transfusions, especially group “O” SDP transfusions, more safe for the patients due to its greater utility for above-cited reasons.

With this intention, we prepared fifty SDP’s from group O donors using a PAS which would reduce the titers of naturally occurring antibodies in the original PLT unit. These SDPs were also compared for quality parameters with fifty SDP’s collected in 100% plasma, over a 5-day storage time. PAS was selected as an isotonic buffering fluid of choice due to its many advantages.[17]

There was a significant reduction in titers of anti-A and anti-B in the study arm SDPs (P < 0.001) when compared to the titers of plasma from pilot tube. The median titers in the study arm dropped from 128 to 16 for anti-A and anti-B antibodies. The maximum antibody titer after PAS addition in any unit was up to 64 [Table 1]. PLTs with high titers of naturally occurring isoagglutinins can pose a risk of hemolysis if units are given across ABO barrier and the risk is greater when group O SDP is released to non-group O patients.[11] The critical titers depend on the methodology used and may be >1:16 for in vitro hemolysis assay, >1:64–1:100 for direct agglutination, and >1:256–1:400 for indirect antiglobulin test. However, past literature that can clearly define clinically critical titer levels is lacking.[16] Based on work of different researchers, most blood banks follow a cutoff ranging from 128 to 250 for defining high-titer units.[6] We found that 56% (28/50) of group O SDPs before PAS addition had titers ≥128. This is higher than other reports which cite the prevalence of high-titer group O donors between 30% and 40%.[18] In our study, PCs with titers ≥128 before addition of PAS showed a significant reduction in titer levels post-PAS with geometric mean titer for anti-A and anti-B coming down to 33.8 and 36.1, respectively. This suggests that substitution of plasma with an additive solution leads to a concomitant reduction in isoagglutinin levels. This decrease in titer may benefit the patients by decreasing the potential for hemolysis if incompatible PLT units have to be issued.[11]

For introducing PAS PC as a routine practice, it is important to ensure that all quality parameters are within acceptable range during the storage period. Studies eliciting sustainability of in vitro parameters of PAS PC are available from across many developed nations.[19] However, there is no published data from our country on PAS SDP. This is the first study from India, delving into the effect of PAS on the quality of apheresis PLTs.

Basic SDP quality indicators such as yield, volume, and PLT concentration were similar in both the arms on day 0 and day 4. Swirling was of maximum gradation in both arms on the days of testing. Reduced swirling may be linked to lower pH values.[20]

All units (study and control arm) were found to be successfully leukoreduced as per standard norms. PLT units are more susceptible to contamination than other blood products because of their storage temperature of 22°C–24°C.[21] All units were culture negative when sampled on day 0.

Morphology score gives an idea about the percentage of PLTs maintaining normal discoid shape and extent of PLT storage lesion.[14] The study arm had better morphology scores on day 4 as compared to the control arm. Morphology score positively correlates with greater hypotonic shock response (HSR) and extent of shape change (ESC).[22] Published reports on PAS (SSP+) containing potassium and magnesium have shown an improvement in the in vitro variables such as ESC and HSR by decreasing glucose consumption and PLT activation.[23]

MPV is known to increase during PC storage and is due to swelling of the PLTs causing disc-to-sphere transformation.[24] We found that MPV increased in both arms on day 4 indicating that PAS could not prevent this shape transformation as found in other studies.[22]

As per national guidelines, the pH should be >6 till the end of storage.[20] This was maintained in both arms in our study. The mechanism of pH decrease in both arms is different. For plasma PC, bicarbonate acts as the principal buffer to maintain pH. In PAS PC, due to only 30% plasma, there are reduced bicarbonate levels, but the buffering action of phosphate and the presence of acetate which provide a sink for hydrogen ions, helps maintain pH.[20]

pO2 levels gradually escalated in both arms due to the superior container material used for PLT storage and corroborated the findings in other studies.[23,24] This has been due to the adequate O2 transport through the advanced PLT storage containers. pCO2 levels in study arm were significantly lower than control arm.
on both test days. This is due to lower concentrations of bicarbonate in PAS PC and the bicarbonate sparing effect of acetate and phosphate.[29]

Glucose levels were lower in study arm on the day of collection owing to reduced plasma content in PC. Lactate levels in both arms increased gradually during storage. The levels were significantly higher in control arm on day 4. The mean lactate levels in control and study arm were 14.17 mmol/L and 11.89 mmol/L, respectively. It has been reported that maintaining lactate levels below 20 mmol/L will allow the pH to remain at optimum levels.[20]

The study arm PCs showed a lower LPR and GCR (P < 0.05); [Table 7]. These findings are consistent with other studies on PAS PC.[27] The ratio of LPR to GCR was significantly higher in the study arm, suggesting a lower consumption of glucose per nanomoles of lactate produced. The LPR and GCR are important while considering the total metabolic profile of the PC. A high GCR can cause an early depletion of glucose with consequent cessation of acid production. The glucose depletion and a continual loss of CO2 through the semipermeable storage bag results in a net loss of free protons and an increase in the pH. On the contrary, a high LPR as seen in the control arm produces more acid which requires buffering by bicarbonates to prevent a sharp decline in pH over storage. Thus, a high GCR and a high LPR are not desirable in PC as both can affect the final quality with regard to pH and cellular functionality.[27]

The limitation of the present study is that ESC, HSR, and PLT aggregometry studies were not carried out, and hence, functionality of PLTs could not be commented upon. Furthermore, the in vitro studies of group “O” PAS PC were not performed; hence, we cannot comment on the recovery and survival of PLTs suspended in PAS.

Conclusion

Group O SDPs can be prepared with PAS leading to a significant reduction of naturally occurring antibodies. The effect of PAS on morphology score at day 4 and on GCR and LPR were significant in the study arm. Remaining quality parameters showed similar findings in the two arms. Patients transfused with PAS units, especially during PLT shortages, which require minor ABO mismatches may benefit from transfusion with a reduced risk of hemolysis compared to units suspended in 100% plasma. PAS units can play a crucial beneficial role in managing PLT inventories in high output centers, wherein minor incompatible PC transfusions are often needed due to constraints in PC supply and more prevalence of high-titer O group individuals.

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Conflicts of interest

There are no conflicts of interest.

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