Original Article

Experience of buffy coat pooling of platelets as a supportive care in thrombocytopenic dengue patients: A prospective study

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Abstract

Random donor platelet (RDP) is not sufficient to improve the platelet count in most thrombocytopenic patients. Single donor platelet (SDP) or buffy coat pooled platelet (BCPP) are the two choices to provide a full therapeutic dose of platelets. However, there are constraints in the preparation of SDP due to stringent donor selection procedure, time required for procedure, and need of special expensive equipments and kits. BCPP is widely practiced, especially in the European countries, since 1995. In India, we decided to adopt the procedure of buffy coat pooling of platelets, especially for economically backward patients and for emergencies. This study was prospectively conducted from September 2009 to September 2010. A total of 129 units of BCPP [tested prior for viral markers by enzyme-linked immunosorbent assay (ELISA) and individual donor nucleic acid amplification test (ID-NAT)] were issued to 129 patients suffering from dengue and were included in this study. For comparison between efficacy of SDP and BCCP, patients were divided into two groups of 50 each. The post-transfusion platelet counts of the patients were noted after 2 hours of transfusion for each type of component. The platelet yield varied from 2.5 to 4.4 $\times$ 10^{11} in BCPP samples. The samples analyzed were sterile without any contamination. The different biochemical parameters were analyzed in detail. The observed post-transfusion platelet recovery and corrected count increment (CCI) at 1 hour and 24 hours after BCPP transfusion were similar to that after SDP transfusion. Hence, we concluded that BCPP can be a low cost alternative to SDP in the times of emergencies like dengue and non-affordability by the patient for SDP.

Key words:

buffy coat, buffy coat pooling of platelets, random donor platelets, single donor platelets

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Introduction

Therapeutic or prophylactic use of platelet concentrates is vital for patients with thrombocytopenia due to dengue fever, intensive chemotherapy, and other illnesses. Dengue is one of the major diseases in and around Delhi. Dengue infections are seen every year, thus making it an endemic disease.[1] The mechanisms underlying the bleeding in dengue are multiple. These are vasculopathy, thrombopathy, and disseminated intervascular coagulopathy. Thrombopathy consists of thrombocytopenia and platelet dysfunction.[2] Bleeding occurs more often in patients with severe thrombocytopenia. High-risk patients having platelet count <20,000/mm^3 and risk of bleeding require urgent platelet transfusion.[3]

Platelet concentrates can be obtained by platelet rich plasma (PRP) method, buffy coat method, and apheresis procedure using cell separator machines.[4-6] New trends in the preparation of platelets showed a shift toward the use of the buffy coat method. It has been suggested that this method causes less platelet activation and damage during platelet preparation. Platelet preparation by buffy coat pooling method is practiced in European countries, US, Latin America, and in some Asian countries.[4]

The buffy coat pooling method of harvesting platelets, which is equivalent to single donor platelet (SDP) apheresis, helps to improve platelet yield, meets emergency requirement for platelet, reduces cost to poor patients, and reduces leukocyte contamination in platelet.[4] In the present study, 129 pooled platelet units were prepared and issued to 129 dengue patients, and a study was conducted on platelet yield, storage parameters, and also on sterility parameters on all issued units. Observational study was also conducted on transfused patients and the post-transfusion platelet increment was compared with that of apheresis platelets.

Material and Methods

Buffy coat pooled platelet (BCPP) preparation

The four buffy coat bags prepared by centrifugation of donated whole-blood were hung vertically from a stand for 4 hours at 22°C until the confirmed reports of ABO blood grouping and transfusion transmitted infections (TTI) by enzyme-linked immunosorbent assay (ELISA) and by individual donor nucleic acid amplification testing (ID-NAT) were obtained. The buffy coat bags were connected serially using sterile connecting device(TSCD II, Terumo medical corp, NJ). One plasma unit (200 ml) of the same blood group

was connected to the top-most bag. Plasma going down due to gravity through each bag (which were connected to each other), rinsed each bag in the process, and got collected in the bottom most bag. The bottom-most bag containing 400 ml of fluid (plasma + 4 buffy coat bags) was centrifuged (Cryofuge 6000i, Heraeus, Thermo Scientific) at 1240 rpm for 10 min. After centrifugation, platelets were separated from platelet pool. The Log 3 leucoreduced platelets were collected in a special type 1000 ml platelet bag and stored at 22°C ± 2°C in platelet agitator/incubator for 5 days; however, BCPP were issued within 24-48 hours of preparation in our study.

Sampling

A total of 10 ml of platelets were collected into sample bag attached to the main platelet bag and separated maintaining the closed system. This sample bag was stored in similar conditions as the main bag and used for assessment of platelet quality. A total of 129 BCPP samples were analyzed on the first, third, and the fifth days of storage for platelet count (using Beckman Coulter Cell counter), glucose, lactate levels, and pH using blood gas analyzer (Nova). Bact-Alert (Biomerieux) was also used to check bacterial contamination. Residual platelet counts after filtration were done by Coulter cell counter, and leucocyte count was done by Coulter cell counter as well as Nageotte chamber.

SDPs were collected using Hemonetics MCS+ and Fresenius COM. TEC machines on healthy donors with hemoglobin >12.5% and weight >50 kg. For comparison between efficacy of SDP and BCCP, two groups were studied (50 patients who were transfused with SDP and first 50 patients with BCPP). The post-transfusion platelet counts of the patients were noted after 2 hours of transfusion for each type of the component. The CCI[7] and post-transfusion platelet recovery (PPR)[7] were calculated as follows:

$$\text{CCI} = \frac{(\text{Post transfusion count} - \text{pre-transfusion count}) \times \text{BSA } (m^2)}{\text{Number of platelets transfused}}$$

$$\text{PPR} = \frac{(\text{Post transfusion count} - \text{pre transfusion count}) \times \text{Total blood volume}}{\text{Number of platelets transfused}}$$

Total blood volume was calculated as 75 ml/kg body weight. The Drugs Controller General of India (DCGI) was intimated about the process of buffy coat pooling of platelets, and we were granted the license for the pooling of platelets by the Drug Controller authorities.

Results

In the present study, most of the patients belonged to the 20-29 years age group, and the median age was 27 years; 93 patients suffering from dengue who were transfused with BC pooled platelets were males, while 36 were females.

Table 1 shows the platelet counts and other biochemical parameters of BCPP platelets from days 1-5 of storage. The platelet count did not change much from day 1 to day 5. The viability and metabolic function of platelets were maintained during the 5 days of storage as depicted by the pH and glucose and lactate levels during the storage.

As seen from Table 2, there was not much variation in the platelet count of BCPP and apheresis platelets. The same holds true for the pH values also. By using platelet filter, the leucocyte counts were reduced to Log 3 (1000 times less than the pre-filtration value, 0.077×10^3 to 0.001×10^3), but the platelet count changed very slightly. Also, there was no bacterial contamination found in both SDP and BCPP samples by Bact-Alert done on days 1, 3, 5, and 7 of collection.

Table 3 shows clearly that, in terms of clinical benefit to the patients, BCPP and SDP are not much different. Average increment in platelet count, CCI, and PPR after BCPP transfusion were comparable to that after SDP transfusion.

Discussion

Usage of various platelet products varies greatly between countries and individual institutions.[4] Singh *et al*.,[5] concluded that, in developing countries, apheresis platelets because of their high cost and more technical expertise required, may be recommended only in selected patients either when PRP platelet concentrates and BCPPs in adequate doses are not available in the inventory mostly due to time constraints. The results of this study provide evidence that the viability and metabolic function of platelets were maintained as depicted by the glucose and lactate levels during the 5-day storage period. Also, the pH range of BC pooled platelets complies with the Council of European Guidelines quality requirements for apheresis platelets (6.4-7.4). The average increment in platelet count 2 hours after transfusion of one unit of BCPP is almost equal to that with the transfusion of one unit of SDP. Although, BCPP transfusion involves exposure to more donors than a SDP product, but all the products were found to be sterile by Bact-Alert and, thus, were comparable to SDP in these terms. Also, BCPP was prepared only after testing of donors for viral infections by both ELISA and ID-NAT, making it further safe for the recipients.

Table 1: Platelet counts and biochemical parameters of BCPP (Average values)

	Day 1	Day 3	Day 5
Platelet count (1011)	3.38	3.36	3.32
pH	7.06	7.03	6.90
Glucose (mg/dl)	353.4	335.1	319.1
Lactate (mmol/L)	11.25	12.69	14.34

Table 2: Comparison of BCPP vs Aphaeresis (Average values, Day 1)

	BC pooled platelets	Apheresis platelets
Platelet count ($\times 10^{11}$)	3.38	3.30
pH	7.06	6.95

Table 3: Comparison of efficacy of BCPP and SDP after transfusion

	Average Platelet increment	**CCI -1 After 2 hour	***PPR % after 2 hour	PPR% at 24 hours
BCPP	32000	7.5	82	46
SDP	34000	7.7	85	52

CCI - Corrected count index, * PPR - Post-transfusion platelet recover

Apheresis procedure requires a donor who meets the criteria required for the procedure and also a special kit that cannot be afforded by those with poor financial backgrounds. Most of the patients at our institution needing platelets are below the poverty line (BPL), and treatment affordability is a major concern. As BCPP preparation does not require any such kit, it thereby reduces the cost of platelet therapy.

The pre-procedure investigations for SDP need to be carried out (for the SDP donor) prior to the procedure. Our long experience of preparing SDP since 1996 reveals that most of the admitted dengue patients are hemorrhagic type with platelets counts <10,000. In these cases, clinicians demand immediate platelet transfusion and it becomes difficult to wait for 7 to 8 hours for the SDP donor to be screened and the procedure to be carried out. It is also not easy to generate adequate voluntary apheresis donors to meet the ever increasing demand for SDP due to reasons like time constraints. Our rigorous efforts to create a pool of voluntary apheresis donors has failed to gain momentum in spite of our sincere efforts, and this forced us to explore the possibility of buffy coat pooling of platelets. In order to improve the day-to-day patient care services, two pooled buffy coat platelets of each group are prepared daily for emergency purposes at our centre.

Keeping in mind the above constraints and limitations and with the aim to serve the needy patients, we took the initiative to carry out the buffy coat pooling of platelets. We have incorporated certain protocols in our institute to provide better services regarding the issue of platelets which is, in dengue crisis, if the demand for SDP comes in the night hours or if the SDP donor is not available, BCPP is issued.

Conclusions

We found BCPP very valuable during the dengue fever season in 2009-2010. BCPP can be a good alternative for SDP for correcting thrombocytopenia in cases of emergencies like dengue, non-availability of donors, and non-affordability of patients for apheresis.

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