Extra-Venous Use of Autologous Platelet Concentrate: Beginning of a New Era of Therapy of Transfusion Medicine?

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Abstract Autologous infusion of blood platelets to induce healing of injured tissue is reported in several recent journals. The presence of many ‘platelet-derived-factors’ forms the basis of these studies. These studies have demonstrated improvement in 70–80% patients over a period of up to several months. We have identified the lapses in their techniques. We decided to undertake a small pilot study to test our use of “platelet concentrate” (U. S. P.) in patients of tendon injuries and also to establish a protocol for the extra-venous use of autologous blood platelets. We present, here, an improved technique of autologous platelet therapy in three groups of patients. Our results are compared with four earlier studies. Enhancement in clinical recovery of patients was achieved in shorter interval of time. We have concluded that use of “Platelet Concentrate” with its quality control tests, seems to be better in place of PRP and/or ‘uncontrolled’ platelet injection. Further, selection of patients for this therapy is crucial. Patients with acute bursitis do not appear ideal for this kind of therapy. However, chronic tendon injuries that are likely to worsen on corticosteroid injections can be treated with autologous platelets with excellent results. This appears to pave a new path for mesodermal regeneration and healing by extra venous use of platelet concentrate.

Keywords Extra-venous · Platelet concentrates · Tendon injuries · Platelet concentrate · Platelet-rich-plasma · Mesodermal healing

Introduction

Use of autologous ‘Platelet-rich-plasma’ (PRP) as a therapeutic agent is first reported by Ferari et al. [1]. Subsequently, PRP is used as extra-venous ‘drug’ in many varied fields including: orthopedics, sports medicine, dentistry, ENT, neurosurgery, ophthalmology and urology, cosmetic, cardiothoracic and maxillofacial surgery [2].

The healing ability of platelets is evidenced by the prompt healing that occurs at the site of phlebotomy. When the same platelets are injected at the site of any other injury, ideally they should have produced far better results than what has been reported in previous studies. This leads us to wonder as to what was lacking in the previous techniques: whether the platelets injected were in viable state or was the platelets preparation and storage at fault? This was the background of present study. A review of previous studies that specify the use of autologous platelets for wound healing was undertaken. Sure enough, the following shortcomings were noted:

- (1) Anti-platelet medications: Most of the studies have not taken care to discontinue all the medicines that could influence the platelet activity.
- (2) Some of the studies were done using ‘autologous platelet-rich-plasma’ (PRP) prepared by using ready-to-use commercial kits. None of the studies mention the precautions used during phlebotomy; time lapse between blood collection and PRP preparation; handling and storage of PRP before injection; etc. all of which are critical for preservation of platelet activity. Further, failure to agitate the platelet suspension will reduce the viability (and, probably, the release of growth factors) [3].

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- (3) In these studies the presence of platelets in the supernatant plasma (which was injected) has been *assumed*, not proven. Similarly, the viability of platelets in the PRP is assumed.

Notes on Use of ‘Platelet Concentrates’ for the Present Study

1. Platelet concentrate and PRP prepared from donor’s blood comes under the purview of food and drug administration (FDA or other authorities) in most countries. These products are subjected to stringent quality control tests before being released for therapeutic use. Although autologous blood or components do not require such legal measures prudence would call for application of such tests even for autologous use. Using ‘platelet concentrates’ instead of PRP and confirming the ‘swirling movement’ before injection ensures that the material injected contains ‘viable platelets’.
2. Observation for ‘Swirling Movements’ in Platelet Concentration is an important quality control test [4]. The suspended viable platelets exhibit swirling movement and are capable of ‘releasing’ their ‘factors’ when used for therapy.
3. It is necessary to recall that ‘release’ of factors from platelets leads to the coagulation-cascade which is completed within minutes and platelet aggregate formation on the endothelial cells seems to be essential in vascular development in regeneration of tissues [5].
4. The platelet granules contain a variety of growth factors [6]. It has been suggested that supra-physiological concentrates of growth factors present in platelet concentrate could work in a positive way by accelerating wound healing, decreasing inflammatory reaction and promoting regeneration rather than repair of the affected tissues [7, 8].

5. The present study was designed to prepare a ‘standard, quality, platelet concentrate’ based on the patient’s body weight; taking all precautions and subjecting it to stringent quality control tests before injection at the site of damaged tissue.

A team of workers from blood bank envisaged this study and collaboration was sought from willing orthopedic surgeons.

Materials and Methods

Patients had presented with tendinopathies at the office practice of the authors. They were offered the autologous platelet injection therapy. All those who opted, were included in this study.

Based on their diagnosis they were segregated into three groups: (Table 1 shows clinical details all groups).

Group I: Four patients with unilateral epicondylitis.

Group II: Two patients with plantar fasciitis.

Group III: Mis-diagnosed tendinopathy. (We have also included two patients with retrocalcaneal bursitis who had presented with signs of severe inflammation and was misdiagnosed as tendinopathy at the onset of the study).

They were assessed on the basis of:

1. *Dolor*—Pain as per visual analogue score (VAS).
2. *Rubor*—Presence of swelling or redness at the epicondylar region.
3. *Functio laesa*—Extent of mobility of the arm or leg graded between I and IV.

The procedure was explained to the patients and their consent was taken. Each patient was examined in detail.

Symptoms, Signs and other relevant objective evidence such as extent of mobility of the limb were recorded (Tables 1, 3; Fig. 1). All the patients were followed up at 4, 8, and 12 weeks.

Table 1 The clinical detail that is the age, sex, duration of illness, and earlier treatment received by the patients

Case no.	Age/Sex	Duration of illness (months)	Presented with	Treatment received before this study
1	52/F	12	Left-Epicondylitis	NSAIDS & 2.inj. of Corticosteroids
2	50/F	3	Left-Epicondylitis	NSAIDS
3	14/F	12	Right-Epicondylitis	NSAIDS
4	50/F	8	Right-Epicondylitis	NSAIDS
5	49/F	12	Right-Plantar Fasciitis	NSAIDS
6	39/M	12	Left-Plantar Fasciitis	NSAIDS
7	65/M	5	Right-Retrocalcaneal Bursitis	NSAIDS & 2.inj. of Corticosteroids
8	50/F	18	Left-Retrocalcaneal Bursitis	NSAIDS & 2.inj. of Corticosteroids

Fig. 1 Graphical presentation of Table 3 Periodic clinical assessment of individual patients

Diagnostic procedures such as X-ray, MRI and USG were performed if required.

Procedure for Platelet Preparation

Stage I

Precautions:

- (1) Withdraw “anti-platelet” drugs 10 days before the procedure.
- (2) Approximately 5% of the body weight is the estimated volume of blood to be withdrawn.

Stage II

Blood Collection

- (3) Select a suitable vein (antecubital or any other).
- (4) Prepare a “double” bag for blood collection. Collect blood using standard procedure.
- (5) Perform a clean phlebotomy complete collection within 10 min.

Stage III

Procedure with refrigerated centrifuge: [Follow the S. O. P. for preparation of “Platelet Concentration” of the blood bank] [Steps 6 to 9].

The remaining blood may be re-infused to the patient.

Stage IV

- (10) Keep the bag containing platelet concentrate at ambient temperature for 30–60 min for “incubation”.

- (11) After incubation, keep the bag on “platelet agitator” for at least 10 min before issuing.

- (12) Inspect the platelet concentrate for “swirling movement” before issuing. Perform the platelet count (Table 2). The person who carries the bag of “platelet concentrate” should be instructed NOT to allow the bag to be refrigerated.

Procedure for Platelet Concentrate Injection

The Procedure is explained to the patient. Consent is obtained. The area to be injected is cleaned thoroughly. Lignocaine is injected into the site selected for injection.

22-gauge needle and 10 ml syringe is used to draw 2–3 ml of platelet rich concentrate and injected into the area of maximal tenderness. A “single skin poke” with “peppering technique” is used.

The patient is kept in sitting position for 15 min immediately after the injection. He/she is asked to limit the

Table 2 This table depicts the quantitative and qualitative status of platelets at the time of injection

Case no.	Blood collected in CPD approximately 5% of body weight (ml)	Platelet concentrate injected	Swirling movement
1	250	10.8×10^9 in 3 ml	Present++++
2	140	8.4×10^9 in 2 ml	Present++++
3	300	11.6×10^9 in 3 ml	Present++++
4	300	10.4×10^9 in 3 ml	Present++++
5	250	13.6×10^9 in 3 ml	Present++++
6	300	12.4×10^9 in 3 ml	Present++++
7	180	10.2×10^9 in 2 ml	Present++++
8	260	10.6×10^9 in 2 ml	Present++++

Swirling movement is graded between + to 4+

use of the limb for 48 h. He/she is given acetaminophen for pain. NSAIDS is prohibited.

The patient is followed up at 4, 8, and 12 weeks.

Results

Table 3 and Fig. 1 depicts the results obtained in all patients included in the study.

Our results showed 100% relief from pain with restoration of mobility in the first and second group. Enhancement in clinical recovery of patients was achieved in shorter interval of time (within 4–12 weeks). The third group of patients showed no improvement.

Discussion

PRP was first used in cardiac surgery by Ferari et al. in 1987 after an open heart surgery. It is now used widely in

diverse fields of medicine to promote healing by natural regeneration of tissue.

In 2003 Edwards and Calandruccio [9] demonstrated that 22 of 28 patients (79%) with refractory epicondylitis were completely free of pain following. Autologous blood injection therapy.

Barret et al. [10] enrolled nine patients in a pilot study to evaluate PRP Injections with plantar fascitis. Six of the nine patients achieved complete symptomatic relief after 2 months. One of the three unsuccessful patients eventually found complete relief following additional PRP injection.

A study carried out by David crane et al. [11] showed that 7 out of 28 patients showed no benefits. The reason documented was small sample size and poor platelet standardization.

Another study by Mishra et al. [12] evaluated 140 patients with chronic epicondylar elbow pain. Of these patients 20 met the study criteria and were surgical candidates who had failed conservative treatments. In total 15 were treated with one PRP injection and five were controls with local anaesthetic. The treatment group noted 60% improvement in 8 weeks, 81% at 6 months, and 93% at final follow up at 12–38 months.

The above discussion is summarized in Table 4.

Conclusion

The analysis of results obtained in present study is as follows:

- (1) Platelet concentrates prepared under expert supervision of blood bank persons shall ensure good count and optimum functional status.
- (2) There is reduced risk of infection as the whole procedure of platelet separation is done under closed system in a quality controlled establishment.

Table 3 The clinical findings based on which the results of the study is recorded

Case no.	Age/Sex	Day 1			Follow up one month			Follow up 2 months			Follow up 3 months			Results %
		A	B	C	A	B	C	A	B	C	A	B	C	
1	52/F	8	II	Absent	6	II	Absent	3	III	Absent	0	IV	Absent	100
2	50/F	7	II	Absent	6	II	Absent	2	III	Absent	0	IV	Absent	100
3	14/F	8	II	Present	5	III	Absent	2	IV	Absent	0	IV	Absent	100
4	50/F	10	I	Present	9	I	Present	6	III	Absent	0	IV	Absent	100
5	49/F	10	II	Present	8	II	Reduced	4	III	Absent	0	IV	Absent	100
6	39/M	9	II	Present	7	II	Reduced	3	III	Reduced	0	IV	Absent	100
7	65/M	10	I	Present	10	I	Present	10	I	Present	10	I	Present	0
8	50/F	10	II	Present	10	II	Present	10	I	Present	10	I	Present	0

A Visual analogue score as observed from day one to follow up for 3 months

B Grade of mobility

C Presence of Swelling and/or redness at the site of tendinopathy

Table 4 Comparison of analysis of results

Author	Patients	Results	Duration in months
Edwards and Calandruccio	28 Epicondylitis	22 (78.5%)	6–24
Barret et al.	9 Plantar fascitis	6 (66.6%)	2
David Crane	28	21 (75%)	Not mentioned
Allan Mishra	20 Epicondylitis	93%	12–38
Present study	8 (6 + 2)	6 (100%)	3

- (3) The patients showed better improvement in terms of relief from pain, restoration of mobility and duration of time required for clinical improvement.
- (4) The patients did not have any side effects.
- (5) These patients did not have recurrence of symptoms when followed up after 5 months of treatment with platelet concentrates injection.

We finally conclude that platelet rich concentrates prepared as per U.S.P with quality control testing is more appropriate for extra-venous use of platelets for tissue healing.

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