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Clinical outcome of post thrombotic syndrome in lower limbs treated by a novel method

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Abstract
Background: Post-thrombotic syndrome (PTS) is a chronic and frequent complication of acute proximal DVT, affecting approximately half of patients within the first 1–2 years. Symptoms vary from mild oedema to chronic pain and ulceration. Severe PTS occurs in 5–10% of all symptomatic DVTs, and may lead to venous ulceration. PTS results in significant disability and impaired quality of life [5] and has a substantial healthcare cost [6]. Recent research has demonstrated that patients with PTS have a poorer quality of life. The signs and symptoms of PTS vary from patient to patient and may include edema, heaviness, leg pain, eczema, hyperpigmentation, lipodermatosclerosis, and venous ulcers.
Methods: Five patients who were diagnosed as PTS were treated in the orthopaedic department with the help of PRP injection locally. The outcomes were measured and documented with the help of villalta score.
Results: All the patients were followed up for a minimal period of 3 months. The VILLALTA score improved considerably in all the patients and the patients were symptomatically better. The majority of the improvement was seen in the swelling and pain which reduced considerably after the infiltration of PRP. The VILLALTA SCORES after infiltration were 6 in one patient and, <5 in the remaining patients.
Conclusion: Although PTS, is a recognised event in many patients of DVT, no gold standard investigation or treatment modality exists till date. Although many physical methods, pharmacological agents, surgeries were tried to effectively treat PTS, none of the methods could stand the test of the time. In comparison to the above methods this method is a relatively easy economical and reproducible way of treating the patients of PTS.

Keywords: Post thrombotic syndrome, villalta score, platelet rich plasma

Introduction
Post-thrombotic syndrome (PTS) is a potentially disabling, chronic complication estimated to occur in 20–67% of adult patients following deep venous thrombosis (DVT) [1, 2, 3] Symptoms vary from mild oedema to chronic pain and ulceration [1, 3].
Post-thrombotic syndrome (PTS) is a chronic and frequent complication of acute proximal DVT, affecting approximately half of patients within the first 1–2 years [4].
Severe PTS occurs in 5–10% of all symptomatic DVTs, and may lead to venous ulceration. PTS results in significant disability and impaired quality of life [5] and has a substantial healthcare cost [6].
Recent research has demonstrated that patients with PTS have a poorer quality of life than patients with chronic lung disease, diabetes, or arthritis [7], and that those with severe PTS have a quality of life comparable to that of patients with congestive heart failure or cancer [5].
The signs and symptoms of PTS vary from patient to patient and may include edema, heaviness, leg pain, eczema, hyperpigmentation, lipodermatosclerosis, and venous ulcers. However, PTS and primary valvular insufficiency share signs and symptoms of chronic venous insufficiency, making correct diagnosis of PTS difficult [8].

Subjects and Methods
The patients were selected from the orthopaedics out patient department who presented with various symptoms of lower limbs which suggested towards a diagnosis of post thrombotic syndrome.

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Inclusion criteria
1. All the patients presenting to Out Patient Department, whose VILLALTA SCORE was >5.
2. Patients with previous history of Deep vein thrombosis.
3. Patients who consented to the procedure

Exclusion criteria
I. Active malignancy.
II. Life expectancy less than 2 years due to any recognised cause.
III. Underlying infection, arterial insufficiency (Fontaine ‡ 2b),
IV. Pregnant patients.

The procedure was explained to the patient and written informed consent of the patient was taken.Approval from the local ethical committee for the treatment was taken. The patients were identified to have post thrombotic syndrome based on Villalta Scale.

Description of the Villalta scale. The Villalta scale was designed specifically for patients with PTS and introduced in an abstract in 1994 as a disease-specific assessment questionnaire for diagnosis and classification of the severity of PTS.

Table 1: Symptoms None Mild Moderate Severe [9]

Symptoms	0 Points	1 Point	2 Points	3 Points
Pain	0	1	2	3
Cramps	0	1	2	3
Heaviness	0	1	2	3
Paresthesias	0	1	2	3
Pruritis	0	1	2	3

Table: Signs

Pretibial edema	0	1	2	3
Skin Induration	0	1	2	3
Hyperpigmentation	0	1	2	3
Redness	0	1	2	3
Ectasia	0	1	2	3
Pain on calf compression	0	1	2	3

Venous Ulcer absent Present
The Villalta scale (Table 1) assesses five symptoms (pain, cramps, heaviness, paresthesia, pruritus) and six clinical signs (pretibial edema, skin induration, hyperpigmentation, redness, venous ectasia, pain on calf compression). Each variable has a 4-point scale ranging from 0 (absent) to 3 (severe). PTS is diagnosed if the Villalta score is >5 or if a venous ulcer is present. A score of 5–9 is categorized as mild, 10–14 as moderate, and >15 as severe disease.

The subcommittee for Control of Anticoagulation of the International Society for Thrombosis and Hemostasis has recommended the Villalta scale as the most appropriate method for diagnosis of PTS [10]. Furthermore, a recent systematic review of various scoring systems for PTS revealed that the Villalta score, combined with a venous disease-specific quality of life questionnaire, was the most suitable gold standard for diagnosis and classification of PTS. [11].

While in some studies two consecutive assessments that corresponded to PTS (i.e. Villalta score ‡ 5) were required to diagnose PTS [12, 13, 14]. in other studies PTS has been diagnosed based on a single assessment [15, 16]. In this study we used a single assessment of villalta score to classify and

diagnose the patients of post thrombotic syndrome.

All the 5 patients were treated with injection of platelet rich plasma (PRP) in to the affected lower limb. The perforators were marked over the skin and PRP was infiltrated in the vicinity of the deep venous system under strict aseptic precautions.

The patients were admitted in the ward and watched for the development of hypersensitivity reactions or any early complications. When no complications were noted, the injections were repeated every 5 days.

A minimum of three PRP infiltrations and a maximum of Five PRP infiltrations were given to the patients.

Weekly follow up of the patient was done and the patients were followed up for a minimum period of three months. Scoring according to VILLALTA scale was done and the serial photographs were taken to monitor the appearance of the affected lower limbs.

Preparation of platelet rich plasma
In the clinical standard setting, blood is drawn from the median cubital vein. When a cell saver is used to manufacture PRP, autologous whole blood is collected into standard donor bags filled by gravity, not exceeding the maximum allowable predonation volume in relation to the citrate volume in the blood bag. When tabletop devices are used, the blood is carefully collected by aspiration techniques into syringes, avoiding "negative pulling" to fill the syringes quickly. The use of a needle diameter larger than 17 gauge avoids trauma to the platelets during the blood draw. The autologous predonated blood is collected in sufficient amounts of anticoagulation citrate dextrose-A solution (ACD-A). In general, a ratio of 1 mL of ACD-A to 7–8 mL of whole blood should be maintained. The aspirated blood is gently agitated to thoroughly mix the anticoagulant with the blood. Currently, most cell savers use a Latham (tapered) bowl instead of a Baylor (straight) bowl, ranging in volume between 50 and 225 mL. Furthermore, continuous autotransfusion systems, not using a bowl, can also be used to prepare PRP.

These sequester the whole blood in a semiautomatic controlled operating mode by centrifugation at 5600 rpm, separating the platelet-poor plasma (PPP) from the buffy coat layer and erythrocytes. The PPP volume is separately collected in a blood bag. Thereafter, centrifugation is slowed down to 2400 rpm to obtain the buffy coat layer consisting of PRP and leukocytes, which is collected in a separate blood bag or syringe. After this procedure, the erythrocytes are also separately collected in a blood bag. The collected PPP and erythrocytes are reinfused during surgery at a time determined by the anesthesiologist. The collected PRP is used to prepare PG for application to tissues. With tabletop devices, a similar protocol of high-and low-speed centrifugation is followed. Depending on the brand of tabletop device, one may collect all blood components separately or collect only PRP. In those cases where no retransfusion of blood components is feasible, the PPP and erythrocytes are discarded. Picture of the PRP machine used is shown below.

Method of injection
Once the PRP was procured, the patients of PTS were infiltrated into the affected lower limb in the following manner. After painting and drapping the affected limb, with the help of compression USG and Colour Doppler, the veins are identified and thrombosed veins re identified by the inability to compress them with the compression.

Ultrasound
With 4cm gap, the prepared PRP will then be infiltrated across the length of thrombosed vein and routine aseptic dressing given for a day.

Literature Review
Pathophysiology of development of Post Thrombotic Syndrome: At the macroscopic level, acute DVT causes partial or complete obstruction of venous outflow. Recanalization, the process by which the thrombus undergoes changes in size, shape, and structure, allows the venous lumen to be re-established. There is an approximately 50% reduction in thrombus load during the first 3 months [17], with recanalization being seen as early as 6 weeks from diagnosis [18]. Importantly, 50% of legs at 3 years still demonstrate some residual thrombus causing partial obstruction. The rate of recanalization appears to be related to the initial thrombus load [19] and thrombus site, with distal thrombi undergoing more rapid and complete resolution [20]. The underlying mechanisms of recanalization are less well understood. It is a complex process involving intrinsic events within the thrombus, as well as systemic activation of fibrinolysis [21]. Killewich *et al*. suggested that regression of an acute DVT is related to an increase in endogenous fibrinolysis [22]. Similarly, a reciprocal relationship between levels of fibrinolytic inhibitors and the degree of recanalization has been described [21]. The inflammatory response to acute thrombosis and the process of recanalization directly damages venous valves, resulting in valvular reflux. Reflux occurs early, with a progressive increase in prevalence from 17% of patients at 1 week to 69% of patients at 1 year after DVT. The degree of initial vein occlusion correlates with the likelihood of developing reflux [23]. Moreover, rapid resolution of the thrombus may preserve valvular function [24]. Reflux, obstruction or a combination of the two results in progressive calf muscle pump dysfunction and venous hypertension. Ambulation and a standing posture sustain the abnormal venous hemodynamics. Ultimately, raised deep venous pressures are transmitted to the capillary beds, promoting transudation of fluid and large molecules, resulting in tissue edema, subcutaneous fibrosis, and, finally, tissue hypoxia and ulceration [25-27].

Risk factors for developing PTS Recurrent DVTs and DVT location
The most significant risk factor for the development of PTS is recurrent ipsilateral DVT, leading to a three-fold to six-fold increased risk [12, 13, 15]. This association is probably attributable to further exacerbation of venous outflow obstruction and increased damage to already compromised valves. The relationship between thrombus location and PTS is an area of continuing research. Several studies have demonstrated an increased risk of proximal DVTs over that of calf or popliteal vein DVTs [15, 28]. It should be noted that proximal DVTs are themselves a heterogeneous group, comprising both iliofemoral and femoropopliteal DVT. This distinction is important, because, in iliofemoral DVTs, the obstruction is often above the entry of the profunda femoral vein, thereby impairing collateral flow. Consequently, iliofemoral DVT confers a greater risk of DVT recurrence [29] and a higher risk of PTS than popliteal DVTs [28].
Venous signs and symptoms associated with distal DVTs may also be less severe than their proximal counterparts [30, 31].
Nonetheless, the impact of distal thrombi should not be underestimated. Approximately 9–20% of below-knee DVTs

will propagate to involve more proximal veins [32].
Improved rates of thrombus regression and recanalization have been demonstrated for calf vein thrombi [34].
This, along with a reduced rate of DVT recurrence, may explain the lower incidence and severity of PTS seen for distal thrombi.
The pathophysiologic mechanism whereby PTS develops after DVT is not fully understood. Clinical observations have shown that a blood clot forming in the deep veins of the lower extremity can cause inflammation and interrupt venous blood flow to the heart, causing severe damage to the vein valves, making them leaky and allowing retrograde blood flow to the ankle. Such venous blood flow interruption and retrograde blood flow can cause venous hypertension, thereby leading to hyperpigmentation, corona phlebectatica, lipodermatosclerosis or ulceration. These processes include leukocyte activation, adhesion and migration through the basement membrane with release of growth factors and proteases [35]. Experimental observations have revealed that leukocytes mediate the release and activation of metalloproteinase 2 (MMP2) and MMP-9, as well as promoting vein wall fibrosis. Vein wall remodeling after DVT is similar to wound healing, and is associated with increased expression of the procollagen gene and total collagen [36].
This is associated with increased early expression of MMP-9, followed by MMP-2 expression and activity after DVT resolution. Another study has shown that post-thrombotic vein wall remodeling is impaired in cystinecystine receptor 7 (CCR7) (/) mice, which have a profibrotic phenotype, is dependent on a thrombotic mechanism, and is mediated by circulating CCR7 (þ) cells [37].
CCR7(þ) signaling may play an important role in positive vein wall remodeling after a thromboembolic event. venous hypertension plays a central role. Incomplete spontaneous recanalization leads to chronic venous obstruction. Venous wall fibrosis and damaged venous valves cause reflux. All this leads to venous hypertension. Venous hypertension in turn causes dilatation of the capillaries and increased endothelial permeability to plasma, proteins, and erythrocytes. Consequences include edema, inflammation, hyperpigmentation of the skin, and eczema or even the development of a venous ulcer. In addition to all of this, venous hypertension causes distension of the deep veins with an increase in valvular incompetence, which is then transmitted to the superficial venous system via the perforating veins (secondary varicosis) [38].

Pathophysiology of the mechanism of action of PRP
Tissue healing is a well-orchestrated and complex series of events involving cell–cell and cell–matrix interactions, with growth factors serving as messengers to regulate the various processes involved. The "tissue healing" process as a whole has to be considered from the point of view of the type of lesion, which will in turn dictate the degree of healing that can be obtained. Activated macrophages migrated at the site of injured tissues release multiple growth factors, including transforming growth factors-α and -β (TGF-α, TGF-β), PDGF, interleukin-1 (IL-1), and fibroblast growth factor (FGF) [62]. Angiogenesis and fibroplasia starts shortly after day 3, followed by the beginning of collagen synthesis on days 3–5. Most of the PGF in the PRP have mitogenic actions that increase the population of healing cells by mitogenesis. However, the action depends on the presence of further differentiated MSCs [63, 64]. The platelets initiate wound repair by releasing locally acting growth factors via α-granules

degranulation, The secretory proteins contained in the α-granules of platelets include platelet-derived growth factor (PDGF-AA, BB, and AB isomers, transforming growth factor-β (TGF-β),
platelet factor 4 (PF4), interleukin-1 (IL-1), platelet-derived angiogenesis factor (PDAF), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), platelet-derived endothelial growth factor (PDEGF), epithelial cell growth factor (ECGF), insulin-like growth factor (IGF), osteocalcin (Oc), osteonectin (On), fibrinogen (Ff), vitronectin (Vn), fibronectin (Fn), and thrombospondin-1 (TSP-1) [65-79]. Activated PRP reduces the transactivating activity of NF-κB, critical regulator of the inflammatory process, and decreases the expression of COX-2 and CXCR4 target genes. By analyzing a panel of cytokines with different biological significance, in activated PRP researchers observed increases in hepatocyte growth factor (HGF), interleukin-4 and tumor necrosis factor-α (TNF-α). HGF and TNF-α, by disrupting NF-κB-transactivating activity, were important for the anti-inflammatory function of activated PRP. The key molecular mechanisms involved in PRP-inhibitory effects on NF-κB activity were for HGF the enhanced cellular IkBα expression that contributed to NF-κB-p65 subunit retention in the cytosol and nucleo-cytoplasmic shuttling and for TNF-α the p50/50 DNA-bin. {Molecular basis of anti-inflammatory action of platelet-rich plasma on human chondrocytes: Mechanisms of NF-κB inhibition via HGF [80].

Observation and Results

The observations of this intervention series are based on 5 patients treated with PRP infiltration into the lower limbs of post thrombotic syndrome. All the patients in this series were males. Majority of the patients were in between third and fifth decade 80% (n=4) with one patient in the sixth decade 20% (n=1).

All the five patients in our series had a history of Deep Vein Thrombosis (DVT) and were still on treatement at the time of intervention, where as the other two had discontinued the treatment and presented to orthopaedics opd with symptoms of persistent pain, swelling and discolouration of lower limb. In this series 4 patients had BMI of >25, 80%(n=4) with average BMI of 27. However one patient had BMI of 23. Two patients in our series had VILLALTA SCORE of 13, 40%(n=2), One patient presented with a score of 16, 20%(n=1) and the other two patients presented with a score of 8 and 10. Two patients among the five had history of Diabetes Mellitus and were on regular treatment. No other co morbidities were present in any of our patients in the series. Four patients received 3 sittings of PRP infiltration every 5th day and one patient received 5 sittings of PRP infiltration every 5th day. All the patients were followed up for a minimal period of 3 months. The VILLALTA score improved considerably in all the patients and the patients were symptomatically better. The majority of the improvement was seen in the swelling and pain which reduced considerably after the infiltration of PRP. The VILLALTA SCORES after infiltration were 6 in one patient and, <5 in the remaining patients.

Discussion

Five patients of Post thrombotic syndrome were treated by PRP infiltartion in this study. Our study showed an inclination of PTS in males. In a similar study done by Gabriel *et al.* there was a male preponderence in the Post thrombotic syndrome in their study [39, 40, 41].

In this study, severe disabling PTS with ulcers was not seen which was similar to other studies conducted by prandoni *et al.* gabriel f *et al.* We also found that almost all the cases in our series were affected by DVT retrospectively and a similar study done by prandoni *et al.* they found that DVT had significant effect on the causation of PTS [4, 12, 13, 39, 41].

Our series had a significant number of patients with increased BMI, raising a suspicion of increased prevalence of PTS in individuals with high BMI. A study done by Ageno *et al.* kahn *et al* and bizulli *et al.* also reported increased prevalence of PTS in indivifduals with high BMI. But the risk in their study disappeared after adjustment for confounding variables, including age, sex, and location of DVT. This contradiction may be explained by small patient numbers [40, 41, 42].

Although the explanation for the association between PTS and obesity is uncertain, obesity is known to be a risk factor for varicose veins and chronic venous insufficiency [43, 44] and it is conceivable that excess body weight might increase venous pressure and promote valvular reflux in already compromised veins. The potential role of weight reduction in the prevention or management of the postthrombotic syndrome should be evaluated.

Post thrombotic syndrome has remained a problem for a long time now with out any refutable and established standard of care. There are many methods of treatment as well as prevention of this syndrome, which were tried and tested in the past. All the methods which were tried had significant side effects as well as patient dissastisfaction because of which the methods went into disrepute. Even today researches are going on to find out a suitable simple and afe traetment method for the PTS. The various methods triued in the past are Elastic CXompression Stockings (ECS). These are non invasive and a simple method of treatment and are usually considered as a first line of treatment. The pressure was kept at 30-40 mm hg. Because of long hours of usage and a mechanical discomfort, some patients might not be complkiant in wearing ECS [45, 46].

Intermittent Pneumatic Compression can also counteract elevated venous pressure in established PTS. In cases where ECS are ineffective, these devices can be used [47, 48, 49].

Various Pharmacologic agents are also being tried in the treatment of PTS. Many studies were done to determine the efficacy of Rutosides, Pentoxifylline, Macronised purified flavonoid fraction(MPFF) and horse chest nut seed extract in the treatment of PTS. None were very effective to be established as a treatment of PTS [50, 51].

Several surgical options were also tried in the treatment of PTS like stent placement for chronic obstructive venous segment, percutaneous prosthetic vein valve placement, venous valve surgery. In patients with unilateral iliac vein obstruction, Palma and Esperon described a femorofemoral crossover bypass using the contralateral great saphenous vein, a long segment of which is mobilized, subcutaneously tunneled to the contralateral side, and anastomosed end-to-side with the common femoral vein on the affected side. If no suitable great saphenous vein was found, ring-reinforced polytetrafluoroethylene prostheses was used. To prevent bypass occlusion, this procedure was often combined with placement of an arteriovenous anastomosis. In the literature, the patency rate was reported to be 70% to 85% (follow-up: 6 to 216 months). In patients with more extensive venous obstructions (bilateral iliac vein obstruction, involvement of the infrarenal vena cava), femorocaval or iliocaval prosthetic bypass is carried out. Endovascular treatment of obstruction/occlusion in 2000, Neglén *et al.* published the first study of the technique of percutaneous transfemoral

recanalization of the iliac venous outflow tract by means of stent angioplasty [26].

Ulcer healing was achieved in 58% of patients. Neglén *et al.* were able to show a significant improvement inquality of life. Complications (chiefly, thrombotic occlusion of the recanalized segment and hematomas at the catheter puncture site) were present. Where recanalization failed, no progression of clinical signs occurred. The AHA gives only a weak recommendation for endovascular treatment (recommendation class IIb, evidence level B).

The stent angioplasty was carried out along the entire post - thrombotically altered iliac and caval segments. Hybrid procedure when inflow is obstructed by postthrombotic trabeculae in the region of the common femoral vein, in addition to stent angioplasty, surgical desobliteration (endovenotomy) of the common femoral vein and the ostia of its tributaries, especially the deep femoral vein, was carried out. This ensures adequate inflow to the recanalized segment of vein and avoids early thrombotic occlusion: after longitudinal venotomy of the common femoral vein, the postthrombotic trabeculae were removed, and the venotomy was closed with a patch plasty. To improve inflow, additionally, an arteriovenous fistula was created between the femoral vein and the superficial femoral vein; the fistula was then closed 6 weeks to 3 months postoperatively, in order to avoid right heart overload (e31–e33). On the basis of the existing studies, according to the AHA, the hybrid procedure may be considered in patients with severe postthrombotic syndrome (recommendation class IIb, evidence level C) (New venous stents The stents usually employed for arterial stent angioplasty (e.g., Wall stents, nitinol stents) are unsuitable vein, as often long segments of the latter are fibrotic and often the vein is externally compressed (e.g., in May–Thurner syndrome).

Venous stents with greater radial force were developed. High flexibility was also required, so that the stents can adapt to the anatomic course of the veins during movement; in the region of the iliac bifurcation, in particular, angulation up to 90° occurs during sitting. Rethrombosis occurred in few patients. These patients were successfully treated endovascularly. Incompetent venous valves in the common or deep femoral vein can be corrected by valve-reconstructing surgery (internal or external valvuloplasty, extravascular banding of the valvebearing segment. Where valves have been irreversibly destroyed, according to AHA recommendations, transplantation of an arm vein or vein transposition can be carried out. If, after successful recanalization of the obstructions, symptoms persist due to reflux because of secondary varicosis of the great saphenous vein, thermal ablation of the incompetent great saphenous vein is a possible approach. Stripping or ligation of perforating veins that are maintaining an ulcer because of reflux is aimed at alleviating symptoms and healing the ulcer.

Only the diseased segments of veins were operated on. Neovalve construction is a potential approach for patients with refractory ulcers. But none of the options could be established as a gold standard in the treatment of PTS [16, 52-61].

Conclusion

Although PTS, is a recognized event in many patients of DVT, no gold standard investigation or treatment modality exists till date. Although many physical methods, pharmacological agents, surgeries were tried to effectively treat PTS, none of the methods could stand the test of the time. In comparison to the above methods our method is a relatively easy economical and reproducible way of treating the patients of PTS. There are certain limitations of our study. First being the number of the patients. Second being the duration of the treatment and follow up which is relatively short. These limitations can be overcome and the effectivenes of PRP IN PTS can be found out byu increasing the sample of the patients and also by increasing the duration of follow up. Once done on a larger sample of patients, this treatment method can be used as an effective and safe method for the treatment of PTS.

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