OUTCOME OF USING PLATELET, PLASMA AND GROWTH FACTORS AS AN ORTHOBIOLOGIC DERIVATIVE TO AVOID INVASIVE SURGICAL PROCEDURES FOR TREATING KNEE OSTEOARTHRITIS AMONG ELDERLY PATIENTS

Thana Turajane, Pamok Saengsirinavin*, Chaivat Sriratanavudhi*, Vorasilp Cheeva-akrapan*, Wanpen Lappaiwong*, Jongjate Aojanepong***

* **Biomedical Technology Research and Development Center, Police General Hospital, Bangkok, Thailand**
****THAI StemLife, Bangkok, Thailand**

Abstract

Background: The application of platelet, plasma and growth factors (PP&GF) is an intra-articular orthobiologic intervention that has been proven to be safe, having less systemic complications compared with conventional treatments and could constitute an option for treating elderly patients with knee osteoarthritis (OA). However, an intermediate result of using PP&GF has yet to be well established.
Objectives: This study aimed to report the survival analysis of 24-month follow-up treatment using PP&GF among elderly patients with knee OA as primary outcome. The secondary outcomes were functional improvement in terms of international knee documentation committee (IKDC) score, Western Ontario and McMaster Universities Osteoarthritis index (WOMAC) score and visual analog scale (VAS) pain score.
Methods: A prospective cohort study was performed among patients with knee OA (Kellgren and Lawrence (KL) grade I-IV), aged more than 65 years who did not respond to conservative treatments. All patients received intra-articular PP&GF treatment and were followed up to 24 months. Primary outcome was recorded as any surgical treatment at any time point post-PP&GF injection. Secondary outcomes including IKDC, WOMAC and VAS pain score were also assessed.
Results: A total of 184 participants were enrolled in this study. The overall survival rate of patients not undergoing any surgical procedures during 24-month follow-up was 87.50%±2.44%. The mean IKDC, WOMAC and VAS pain scores were 39.59±0.58, 55.9±1.09 and 6.63±0.13, respectively at baseline while those at 24-month follow-up were 46.77±0.81, 38.32±1.33 and 4.92±0.13, respectively. The mean platelet concentrations before and after centrifugation were $1.85x10^5$ cells/μL ($1.20\text{-}3.36x10^5$ cells/μL) and $1.4x10^6$ cells/μL ($5.80x10^5\text{-}3.5\ x10^6$) cells/μL, respectively, which showed final PP&GF products contained 6-10 times higher platelet concentration than those in the peripheral blood.
Conclusion: Intra-articular injection of PP&GF is a potential treatment for severe knee OA especially among elderly patients. This method provided 87.50% survivorship from surgical intervention at 24-month follow-up. Most patients improved both pain and functional outcomes. We propose that the optimal preparation technique for PP&GF is the key step for improving patients' clinical outcomes and regaining their quality of lives.

Keywords: Orthobiologics, Plasma, Platelet, Growth factor, Knee osteoarthritis

J Southeast Asian Med Res 2022**:** 6**:**e0105
https://doi.org/10.55374/jseamed.v6i0.105

Correspondence to:
Cheeva-akrapan V, Biomedical Technology Research and Development Center, Police General Hospital, Bangkok, Thailand
E-mail: vorasilp.c@gmail.com

Received: 13 September 2021
Revised: 27 January 2022
Accepted: 22 February 2022

Introduction

Knee osteoarthritis (OA) is a degenerative health condition impacting nationwide health care systems and health socioeconomic costs and affecting daily life activities of individuals.[1] Patients with knee OA are usually treated in a stepwise pattern starting from patient education, oral medication, physical therapy, intra-articular injection of anti-inflammatory drugs or visco-supplementation agents and surgery. However, conventional modalities have been proven to produce some disadvantages. Oral glucosamine supplement was proven to have insufficient evidence of effectiveness.[2] Bruyere et al. found that 6.3% of patients receiving glucosamine supplement underwent total knee replacement after 8-year follow-up, compared with 14.5% in the placebo group.[3] Intra-articular steroid injection may have short term effect and negatively affects the cartilage.[2, 4] Regarding injecting viscosupplementation agents, Boutefnouchet et al. reported a 67% survival rate at 5 years for patients treated with viscosupplement for knee OA.[5] Patients undergoing total knee arthroplasty (TKA) may need revision surgery due to infection or mechanical loosening over time. [6]

Studies showed that the number of TKAs is rising. One study estimated the amount of TKA in the US alone was predicted to be 1,272,000, 1,921,000 and 3,416,000 in 2025, 2030 and 2040 compared with 688,000 TKAs in 2009.[7] These high number of operations would become problematic considering socio-economic costs.

One of the most vulnerable groups to develop knee OA is advanced age patients. Most have multiple co-morbidities and take multiple medications. For these people, receiving nonsteroidal anti-inflammatory drugs (NSAIDs) for pain relief significantly increased the risk of gastro-intestinal and cardiovascular events and drug interactions including anticoagulants which in turn might affect coagulation cascades.[8] Moreover, some patients might also be categorized as high risk to develop peri-operative myocardial infection or death. Undergoing surgical operation would be a major concern. Treating elderly patients with these modalities might be unsuitable.

Recently, orthobiologic agents including platelet-rich products have gained popularity for treating patients with knee OA due to their biosafety, simplicity and clinical effectiveness. The component of platelets, plasma and growth factors (PP&GF) constitute one of the platelet-rich products adjusted for platelet and fibrin concentrations, leukocyte population and proper activator status. PP&GF initiates the repair processes, modulates inflammation, transports growth factors and attracts medical signaling cells (MSCs).[4, 9] PP&GF differs from platelet rich plasma (PRP) in terms of reproducing substantial amounts of platelet concentration. The PP&GF system provides an average of 6 times higher platelet concentration than baseline value.[10] With this high concentration of platelets in platelet-rich derivative products, large amount of proteins and growth factors are released from platelet alpha granules and act directly to create great healing potential. Up until today, no major adverse events have been reported from PP&GF use.[2, 10] However, the intermediate outcome of PP&GF use in knee OA has not been established. This study aimed to report primary outcome in terms of survival analysis of 24-month follow-up treatment using PP&GF especially among elderly patients with knee OA. Secondary outcomes aimed to assess functional outcomes in terms of international knee documentation committee (IKDC) score, Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) score as well as visual analog scale (VAS) pain score.

Methods

From February 2018 to June 2019, a prospective cohort study was conducted after the internal review board and hospital ethics committee approval at the Biomedical Technology Research and Development Centre, Police General Hospital, Bangkok, Thailand. Calculation of sample size was performed based on a related study [11] with an alpha of 0.05 and a power of 80%. The required sample size was 524 knees divided to 424 knees in Kellgren and Lawrence (KL) grade I to III and 100 knees in KL grade IV groups.

Patients with knee pain visiting the outpatient Orthopaedic Clinic were evaluated clinically and radiographically for their eligibility. Those meeting the following criteria were included in the study: patients aged more than 65 years diagnosed with knee OA classified as KL grade I to IV, those who failed conservative treatments including no response to oral medications for at least six months, physiotherapy for at least three sessions, intra-articular injection of steroid or hyaluronic acid at least one dose, hemoglobin (Hb) levels >11 g/dL, and platelet count >80,000 cells/μL.

The exclusion criteria included patients receiving a diagnosis of meniscal or knee ligament injury, inflammatory arthritis including uncontrolled bleeding disorder, those presenting radiographic deformity of the tibiofemoral angle more than 5 degrees and those having history of using NSAIDs within 5 days of blood drawn or taking anticoagulant or anti-aggregate drugs.

In this study, a total number of 186 patients met the criteria. Patients were informed about the study protocol and signed written consent forms (**Figure 1**).

PP&GF preparation

A 30-ml peripheral blood sample for single knee injection (60 mL for bilateral knees) was collected from each patient. The first 20 mL of blood was separated in 2 centrifuge tubes (red) (PP&GF, Bangkok, Thailand) with 10 mL for each tube to mix with acid citrate dextrose anticoagulant. The blood was centrifuged using the ALPAS centrifugation machine (Bangkok, Thailand) at 250g for 6 minutes. After the first spin, the blood was separated in three components: red blood cells on the bottom layer, a buffy coat in the middle layer and platelet-containing plasma at the top layer. The upper two layers were gently aspirated and transferred to a new tube (yellow) and centrifuged again at 1000g for 10 minutes. After the second spin, the platelet-poor supernatant plasma was gently aspirated for removal. One milliliter of residual leukocyte-rich PP&GF was collected for complete blood count. The remaining PP&GF was aspirated using a 5-mL sterile injection syringe for intra-articular injection. The remaining 10 mL of peripheral blood was transferred to the last tube (green) and was centrifuged once with 250g for 6 min to produce platelet-rich fibrin and used as a natural activator (**Figure 2**).

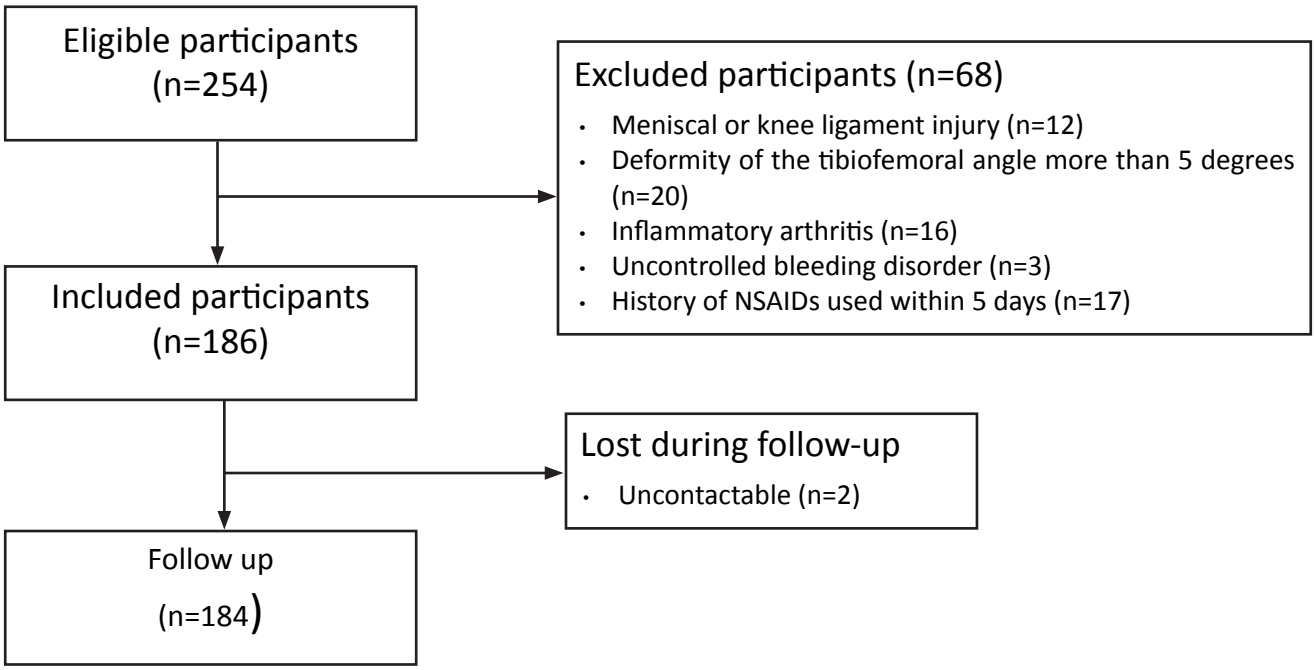

Figure 1. Flow chart of patient enrollment

Figure 2. Platelet, plasma and growth factors (PP&GF) tubes: the red tube is used for the first spinning process. The yellow tube is used for the second spinning process. The green tube is used for platelet-rich fibrin preparation.

Injection protocol

All injections were administered by a well-trained team of orthopedic staff and residents. The patient was placed in the supine position with knee flexed 90 degrees. The injection site was marked at the anteromedial joint space, and an antiseptic agent was applied to the skin. The analgesic agent infiltrated the skin and subcutaneous tissues surrounding the injection site using a 25G needle. Air-test was performed with a 18G needle to confirm the intra-articular placement of the needle. PP&GF biologic agents were delivered to the joint space using a 18G needle. The knee was immediately extended after the biologic agent was delivered, and the patient was allowed to walk in full weight bearing after injection.

Rehabilitation protocol

The patient was instructed to perform fixed arc quadriceps exercise two days after injection. The patient sat on a chair with their legs extended forward for 100 seconds on each side. This exercise was recommended to perform three to five times daily.

Follow-up assessment

Patients were assessed at 2, 4, 6, 12, 18 and 24-month posttreatment follow-up. Primary outcome was recorded as any surgical treatment performed by any surgeon encountered by participants (either arthroscopic knee surgery, unicondylar arthroplasty or TKA) at any time point post PP&GF injection. Secondary outcomes, including IKDC, WOMAC and VAS pain scores, were also assessed.

Statistical analysis

Continuous data were elaborated in mean and standard deviation, and discrete data were elaborated in percentage and proportion. A box plot was used to demonstrate the tendency of collected data. Survival analysis, using monthly interval units, determined the event rate. The time at risk comprised the number of months that the participants were followed up with an end point of 24 months. Right truncation, left truncation, right censoring and left censoring protocol were used. Kaplan-Meier curve was used to evaluate the primary outcome as the percentage of survival at each time point. The difference of survival function was determined using the log-rank test for equality of survivor function. A p-value <0.05 was considered statistically significant. Stata Software, Version 16.0 (Stata Corp, TX, USA) was used for statistical analysis.

Table 1. Characteristics of patients categorized based on KL classification

	KL I	KL II	KL III	KL IV	Total
Amount (knees)	3	67	41	73	184
Age (Mean±SD)	66.67±1.2	73.36±0.66	72.15±0.8	73.5±0.66	73.03±5.45
Sex (Female:Male)	1:2	38:29	31:10	62:11	132:52
BMI (Mean±SD)	27.33±3.53	26.06±0.65	25.05±0.77	25.95±0.6	25.8±5.17
Site (Left:Right)	2:1	33:34	18:23	31:42	84:100

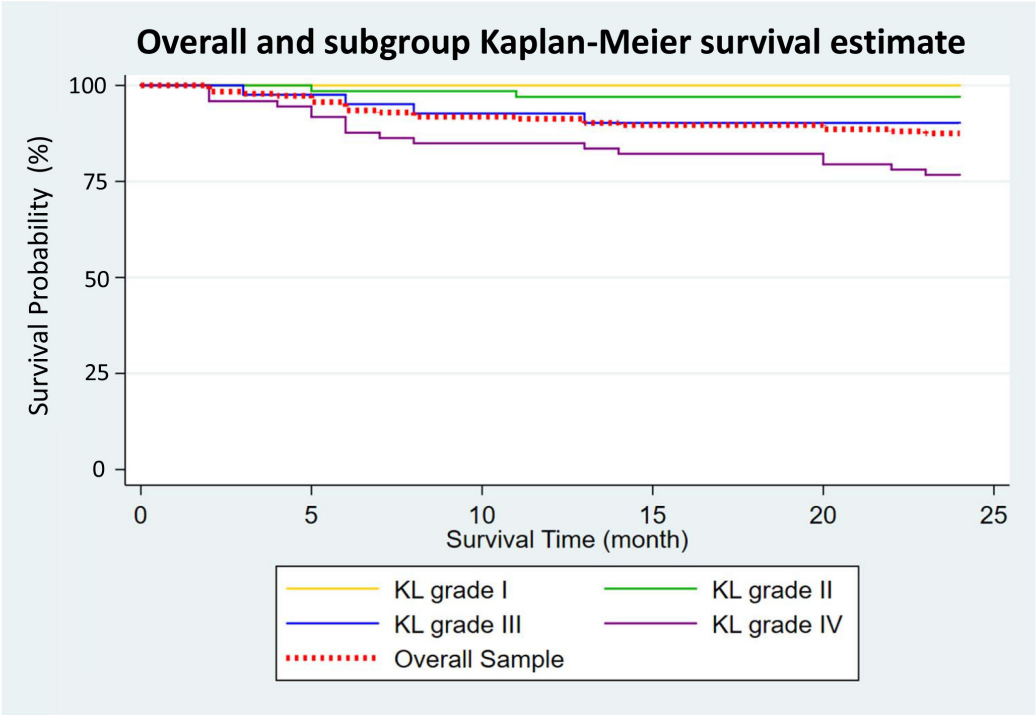

Figure 3. Kaplan-Meier curve showing overall and subgroup analysis of survival estimate rate of participants with different severity of knee OA not undergoing any surgical intervention after intra-articular PP&GF treatment. The red dot line represents overall samples. The yellow line represents KL grade I subgroup. The green line represents KL grade II subgroup. Blue line represents the KL grade III subgroup. Purple line represents the KL grade IV subgroup.

Results

A total number of 186 participants were included. Of these, 2 participants were lost to follow up, leaving 184 participants completing the study. Characteristics of patients are demonstrated in **Table 1**.

The overall survival rate of knees not undergoing any surgical procedures during the 24-month follow-up was 87.50 ± 2.44%. Subgroup analysis for survival rate was performed according to severity grading (KL classification) (**Figure 3**). The log rank test was used to analyze the difference of survival rates between subgroups. The survival rates at 24-month follow-up revealed significant differences between knees with KL I to III and KL grade IV, i.e., 94.59% ± 2.15 and 76.71% ± 4.95, respectively (p = 0.0003). The survival rates at 24-month follow-up also significant differed between knees without subluxation and knees with subluxation, i.e., 90.12% ± 2.34 and 68.18% ± 9.93, respectively (p = 0.0028). The survival rates at 24-month follow-up of knees with KL-IV statistically differed; however, no significance was found between knees without

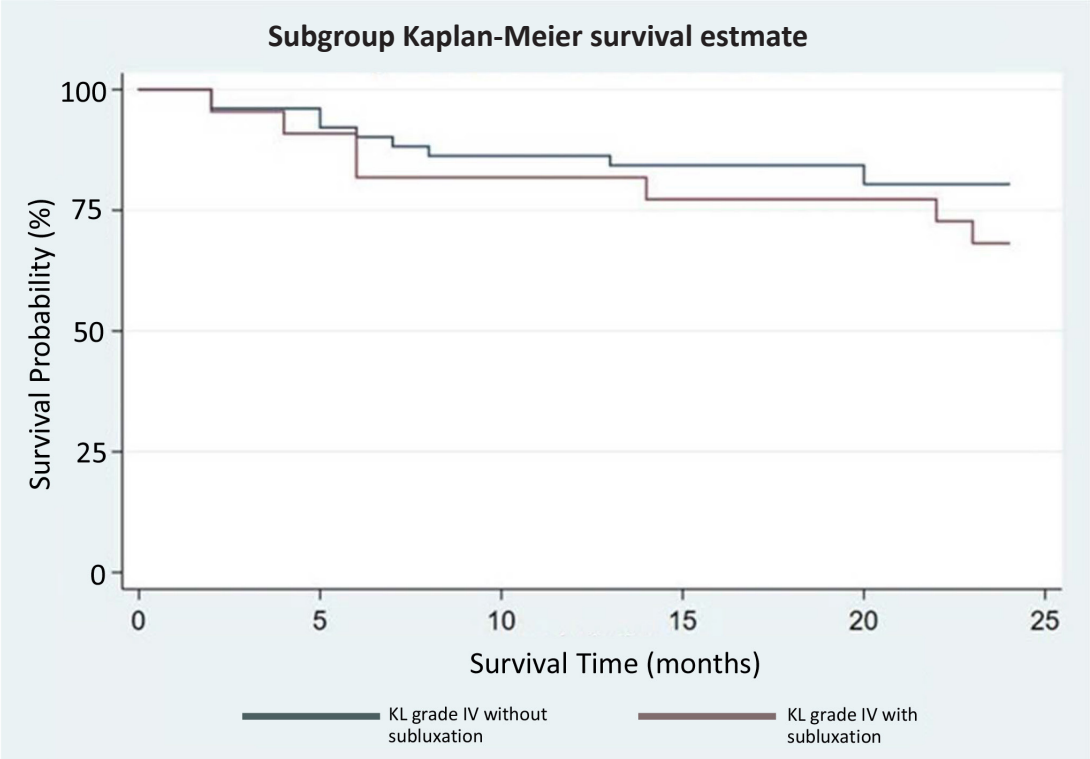

Figure 4. Kaplan-Meier curve showing subgroup analysis of survival estimate rate of participants in knee KL IV with different subluxation profiles not undergoing any surgical intervention after intra-articular PP&GF treatment. The dark green line represents the knee in KL grade IV without subluxation subgroup. The brown line represents the knee in KL grade IV with subluxation subgroup.

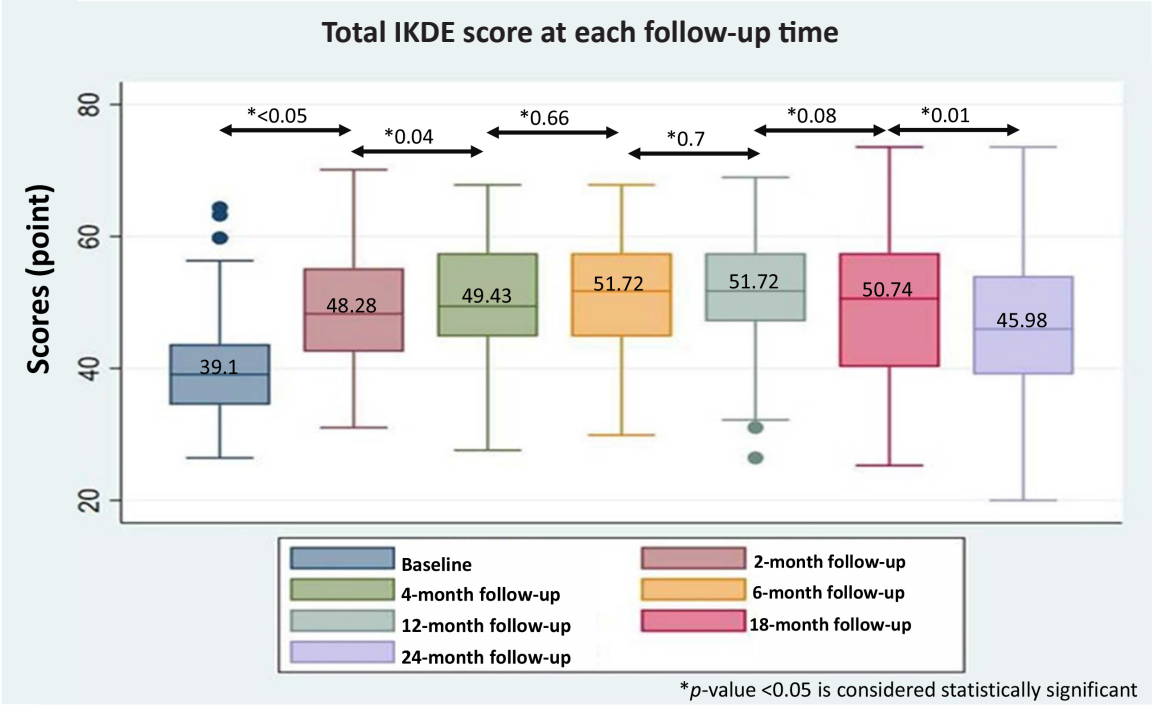

Figure 5. Box plot showing total IKDC score at different follow-up times. The asterisk (*) represents *p*-value of the test of difference between mean score at each follow-up time. The blue box represents score at baseline. The red box represents score at 2-month follow-up. The green box represents score at 4-month follow-up. The orange box represents score at 6-month follow-up. The mint-green box represents score at 12-month follow-up. The pink box represents score at 18-month follow-up. The purple box represents score at 24-month follow-up.

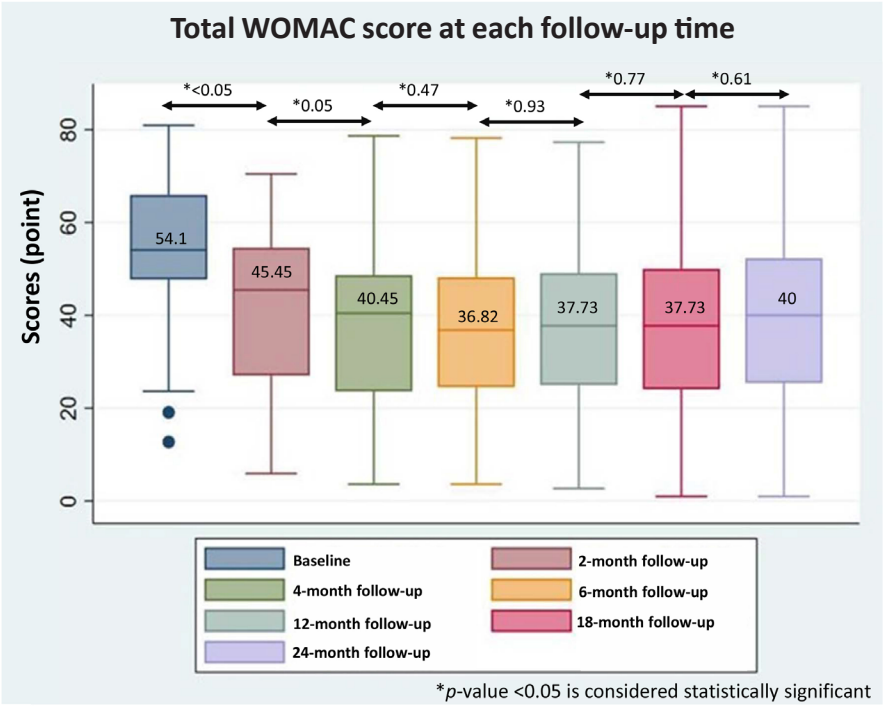

Figure 6. Box plot showing total WOMAC score at different follow-up times. The asterisk (*) represents *p*-value of the test of difference between mean score at each follow-up time. The blue box represents score at baseline. The red box represents score at 2-month follow-up. The green box represents score at 4-month follow-up. The orange box represents score at 6-month follow-up. The mint-green box represents score at 12-month follow-up. The pink box represents score at 18-month follow-up. The purple box represents score at 24-month follow-up.

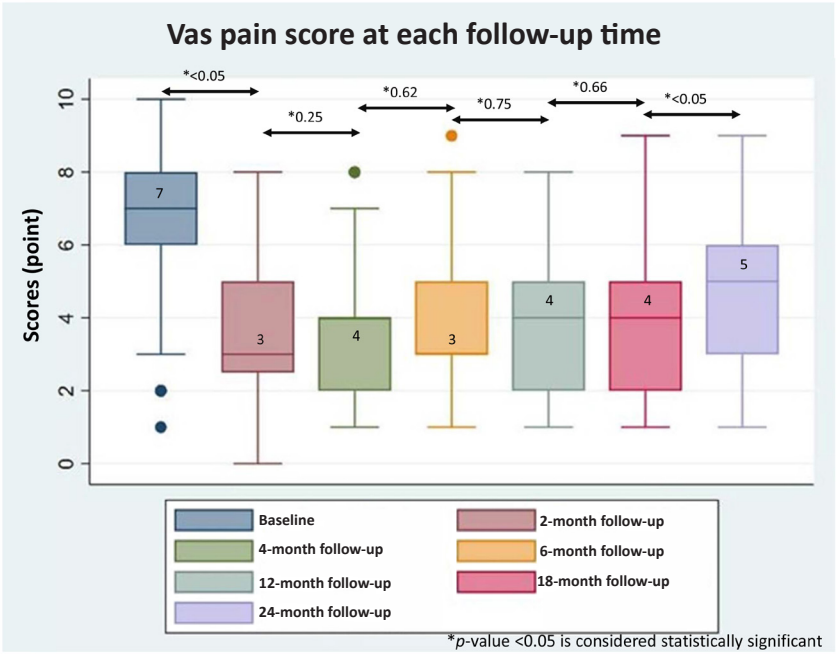

Figure 7. Box plot showing VAS pain scores at different follow-up times. The X axis represents time while the Y axis represents VAS pain scores in point unit. The asterisk (*) represents *p*-value of the test of difference between the mean scores at each follow-up time. The blue box represents the baseline score. The red box represents score at 2-month follow-up. The green box represents score at 4-month follow-up. The orange box represents score at 6-month follow-up. The mint-green box represents score at 12-month follow-up. The pink box represents score at 18-month follow-up. The purple box represents score at 24-month follow-up.

subluxation and knees with subluxation, i.e., 80.39% ± 5.56 and 68.18% ± 9.93, respectively (p= 0.2762) (**Figure 4**).

Regarding total IKDC score, mean IKDC score at 24-month follow-up was 46.77 ±0.81 compared with 39.59 ± 0.58 at baseline. Significant differences were observed between the mean score at baseline and 2-month follow-up, between 2- and 4-month follow-up and between 18- and 24-month follow-up (p <0.05, 0.04, 0.01, respectively). No significant difference of mean scores was observed between each period from 4- to 18-month follow-up (**Figure 5**). IKDC scores, recorded among different follow-up periods were also categorized using KL classification as shown in **Table 2**.

Regarding total WOMAC score, mean WOMAC score at 24-month follow-up was 38.32 ± 1.33 compared with 55.9 ± 1.09 at baseline. A significant difference of mean score was observed between baseline and 2-month follow-up (p <0.05). No significant difference of mean score was observed between each period from 2- to 24-month follow-up (**Figure 6**). WOMAC scores among different follow-ups were also categorized using KL classification shown in **Table 3**.

Regarding VAS pain score, mean VAS pain score was 4.92 ± 0.13 at 24-month follow-up compared with 6.63 ± 0.13 at baseline. Significant differences were noted of mean score between the baseline and at 2-month follow-up (p <0.05) and between 18- and 24-month follow-up (p <0.05). No significant difference was found of the mean score between each period from 4- to 18-month follow-up (**Figure 7**). VAS pain scores during different follow-ups were also categorized using KL classification as shown in **Table 4.**

The mean platelet concentrations before and after centrifugation were $1.85x10^5$ cells/μL ($1.20\text{-}3.36x10^5$ cells/μL) and $1.4x10^6$ cells/μL ($5.80x10^5$-$3.5\ x10^6$) cells/μL, respectively, showing that final PP&GF product increased platelet concentration 6 to 10 times higher than those in peripheral blood samples.

Table 2. Mean IKDC scores recorded among different follow-up periods categorized using KL classification

	KL I	KL II	KL III	KL IV	Total
Baseline	48.28±8.07	40.30±0.94	40.26±1.22	38.58±0.92	39.59±0.58
2-month follow-up	55.56±7.75	48.70±1.00	49.40±1.25	49.02±1.05	49.00±0.63
24-month follow-up	47.24±9.10	45.21±1.22	43.06±1.70	50.34±1.29	46.77±0.81

Table 3. Mean WOMAC score at different follow-ups categorized by KL classification

	KL I	KL II	KL III	KL IV	Total
Baseline	44.85±16.24	53.17±1.81	56.26±2.24	58.47±1.64	55.90±1.09
2-month follow-up	29.39±12.71	41.97±1.95	39.94±2.33	41.24±1.98	41.22±1.18
24-month follow-up	26.52±13.21	41.95±2.10	42.74±2.62	32.42±2.11	38.32±1.33

Table 4. Mean VAS pain scores recorded among different follow-ups categorized using KL classification

	KL I	KL II	KL III	KL IV	Total
Baseline	5.33±2.33	6.19±0.23	6.82±0.28	6.93±0.19	6.63±0.13
2-month follow-up	3±1.53	3.45±0.20	3.97±0.30	3.88±0.22	3.74±0.13
24-month follow-up	5.67±1.45	5.05±0.22	5.53±0.26	4.43±0.19	4.92±0.13

Discussion

In this study, the results showed significantly different survival rates between those with the knee in KL I to III including KL IV with PP&GF treatment. These findings imply an advantage of early intervention in OA knee treatment contributing to reduced surgical interventions. We also found that the non-subluxated knee had a significantly higher survival rate when compared with the knee with subluxation; this implied that mechanical parameters should also be considered along with biologic profiles of knee pathology to select appropriate treatment options. Thus, we proposed that severity of knee OA and subluxation profile are two identifiable negative predictive factors for failure of biologic intervention. However, due to using a small sample size in this study, significant differences of survival rate were not found between the knee with and without subluxation in the KL IV group.

Platelet-rich products entered the spotlight of orthopedic surgeons due to their potential in wound healing and tissue regeneration processes. Once properly activated, alpha granules in platelets released large pool of proteins and growth factors. These growth factors included platelet-derived growth factor (PDGF) promoting mitogen for connective tissue cells, transforming growth factor-β (TGF-β) stimulating osteoprogenitor cells to proliferate and halting the process at later stage of cell differentiation and mineralization. Moreover, insulin-like growth factor (IGF-1) promotes the late stage differentiation and activity of osteoblasts, and vascular endothelial growth factor (VEGF) induces endothelial cell proliferation and migration.[12] These molecular findings explained why PP&GF is suitable to treat knee OA. In this study, the authors chose PP&GF to treat knee OA because it exerts an action on OA at the accurate sites of pathology including chondrocyte, synovium and synovial fluid via its chondrogenic effects, stem cell migration and healing cascade while other traditional treatments mainly focused on eliminating pain. Gobbi et al. studied the effectiveness of intra-articular platelet-rich product injections among patients with symptomatic knee OA and revealed significant improvement in decreasing pain, improving quality of life and returning to their daily life activities after 6 and 12 months.[13] In 2013, Turajane et al. combined intra-articular autologous activated peripheral blood stem cells (AAPBSC) with growth factor addition/preservation (GFAP) along with hyaluronic acid (HA) together with arthroscopic micro-drilling mesenchymal cell stimulation (MSC)[14, 15]; this pilot study highlighted future research to focus on combining different orthobiologics products to maximize the effective use of autologous biomaterials.

Regarding pain reducing mechanisms of platelet-rich derivatives, Lee et al. investigated cannabinoid receptor gene expression in PRP-containing hydrogels used for articular cartilage defects. Cannabinoid receptors CB1 and CB2 have been reported to directly correlate with analgesic effects in animal arthritis models. The result showed that PRP-containing hydrogels produced an increased expression of cannabinoid receptors compared with PRP-free hydrogels.[16] Sundman et al. also found that PRP decreased the concentration of tumor necrosis factor (TNF)-α in synovium and cartilage culture medium. TNF-α is recognized as a pro-inflammatory cytokine initiating neuropathic pain pathways in human disease states.[17]

Regarding functional improvement among patients with OA treated with PRP, Sundman et al. also reported that PRP significantly decreased matrix metalloproteinase-13 (MMP-13) expression in synoviocyte culture media. MMP-13 has been recognized as the cartilage matrix degradation of the synovial membrane contributing to disruption of nutrition, removal of waste products, shock absorption, lubrication mechanisms of the synovial membrane which in turn are responsible for functional outcome of patients.[17]

According to the results of secondary outcomes, a remarkable trend in outcome was observed. The scores were significantly improved at 2-month follow-up after treatment. Comparing the scores at each subsequent follow-up, most were not significantly changed. However, scores once again dropped statistically at 18- and 24-month follow-up. The difference in tendency of outcomes between the 18- and 24-month follow-up was also observed by Filardo et al.[18]

With these trends, we could imply that PP&GF exerted its highest function at 2 to 18 months lasting until 24 months. Thereby, we suggested that a single dose of PP&GF was sufficient to treat knee OA and patients initially responding to intra-articular PP&GF treatment should receive a repeated dose between 12 and 18 months after the first injection to maintain high efficacy of the treatment. Vilchez-Cavazos et al. also performed a meta-analysis to compare the clinical effectiveness of single and multiple injections of platelet-rich derivatives to treat patients with knee OA. The results showed that a single injection was as effective as multiple injections in reducing pain. Multiple injections were more effective in joint functionality than a single injection at 6 months. [19]

Regarding safety issues, in our recent study, after 24-month follow-up of intra-articular PP&GF injections, neither local nor systemic adverse effects were reported. The data correlated with a systematic review of 29 articles by Laver et al. [20] studying platelet-rich product use for OA revealing no adverse effects of intra-articular PRP injections. This emphasized the intermediate term safety profile of intra-articular PP&GF injections and supported the results of our related study on short term safety profiles. [11]

Until now, different preparation techniques and components of platelet-rich products have yielded different patient's outcome. [21] The strength of this study was the protocol for intra-articular PP&GF injection including strict patient selection criteria, reproducible PP&GF preparation technique and using an accurate biologic treatment delivery method. This study comprised a unique and precise method that minimized the heterogeneity of the treatment and uniformly intervened with patients. In this study, the PP&GF preparation technique provided 6 to 10 times higher platelet concentration when compared with those in peripheral blood. We adopted the air-test technique when performing intra-articular injection among all patients receiving PP&GF treatment to ensure that the biologic product was delivered to a correct position in the knee joint.

This study encountered several limitations. First, a small sample size affected the process to prove significant differences among subgroup analysis of the knee with KL IV. Second, the placebo effect of an intra-articular injection could have contributed to positive responses of patients. Nonetheless, Vannabouathong et al. performed a meta-analysis and found that intra-articular PRP had the greatest effect estimates among many treatment modalities when accounting for the intra-articular placebo effect. [22] Third, no information of treatment with repeated dose of the intra-articular PP&GF was reported in this study because we found the trend of secondary outcome after the study ended. Fourth, the primary outcome as decisions of patients to received surgery involved confounders which could have led to bias in this study. Fifth, exploring biochemical profiles of growth factors and protein components of PP&GF was not performed in this study. Lastly, analysis of blood samples other than platelet concentration before and after centrifugation was not performed. Thus, future research focusing on biochemical profiles of leukocyte and red blood cell components is recommended.

Conclusion

An intra-articular injection of PP&GF is a potential treatment for the severe knee OA especially among elderly patients, providing survivorship from surgical intervention at 24 months for 87.50% of patients. Most patients improved in pain and functional outcomes. We propose that the optimal preparation technique for PP&GF is the key step for improving clinical outcomes and quality of life.

Funding

The study was financially supported by the "Police General Hospital Foundation under the Royal Patronage".

References

1. Safiri S, Kolahi A- A, Smith E, Hill C, Bettampadi D, Mansournia MA. Global, regional and national burden of osteoarthritis 1990-2017: a systematic analysis of the global burden of disease study 2017. Ann Rheum Dis 2020; 79: 819-28.

2. Eilik H, Dogu B, Yilmaz F, Begoglu FA, Kuran B. The efficiency of platelet-rich plasma treatment in patients with knee osteoarthritis. J Back Musculoskelet Rehabil 2020; 33: 127-38.
3. Bruyere O, Paveka K, Rovati LC, Gatterova J, Giacovelli G, Olejarova M, et al. Total joint replacement after glucosamine sulphate treatment in knee osteoarthritis: results of a mean 8-year observation of patients from two previous 3-year, randomized, placebo-controlled trials. Osteoarthritis Cartilage 2008; 16: 254-60.
4. Robinson DM, Eng C, Makovitch S, Rothenberg JB, DeLuca S, Douglas S. Non-operative orthobiologic use for rotator cuff disorders and glenohumeral osteoarthritis: a systematic review. J Back Musculoskelet Rehabil 2021; 34: 17-32.
5. Boutefnouchet T, Puranik G, Holmes E, Bell KM. Hylan GF-20 Viscosupplementation in the treatment of symptomatic osteoarthritis of the knee: Clinical effect survivorship at 5 years. Knee Surg Relat Res 2017; 29: 129-36.
6. Delanois RE, Mistry JB, Gwam CU, Mohamed NS, Choksi US, Mont MA. Current epidermiology of revision total knee arthrtoplasty in the United States. J Arthroplasty 2017; 32: 2563-8.
7. Singh JA, Yu S, Chen L, Cleveland JD. Rates of total joint replacement in the United States: future projections to 2020-2040 using the national inpatient sample. J Rheumatol 2019; 46: 1134-40.
8. Turajane T, Wongbunnak R, Patchratrakul T, Ratansumawong K, Poigampetch Y, Songpatanasilp T. Gastrointestinal and cardiovascular risk of non-selective NSAIDs and Cox-2 inhibitors in elderly patients with knee osteoarthritis. J Med Assoc Thai 2009; 92.
9. Carr II JB, Rodeo SA. The role of biologic agents in the management of common shoulder pathologies: current state and future directions. J Shoulder Elbow Surg 2019; 28: 2041-52.
10. Turajane T, Saengsirinavin P, Sriratanavudhi C, Larpaiwong W, Aojanepong J. A prospective, randomized, controlled trial comparing clinical outcomes of intraarticular platelet plasma concentrate and growth factors versus corticosteroid injections in the treatment of knee osteoarthritis. Bank Med J 2021; 17.
11. Turajane T, Sriratanavudhi C, Saengsirinavin P, Chaweewannakorn U, Larpaiwong W, Aojanepong J. Safety and clinical efficacy of platelet rich growth factors (PRGF) in managing knee osteoarthritis after failed conservative treatment: evidence from real practices. J Southeast Asian Med Res 2019; 1: 1-7.
12. Anitua E, Sanchez M, Orive G, Andia I. The potential impact of the preparation rich in growth factors (PRGF) in different medical fields. Biomaterials 2007; 28: 4551-60.
13. Gobbi A, Karmatzikoz G, Mahajan V, Malchira S. Platelet-rich plasma treatment in symptomatic patients with knee osteoarthritis: preliminary results in a group of active patients. Sports Health 2012; 4: 162-72.
14. Turajane T, Chaweewannakorn U, Larbpai boonpong V, Aojanepong J, Thitiset T, Honsawek S et al. Combination of intra-articular autologous activated peripheral blood stem cells with growth factor addition/ preservation and hyaluronic acid in conjunction with arthroscopic microdrilling mesenchymal cell stimulation improves quality of life and regenerates articular cartilage in early osteoarthritic knee disease. J Med Assoc Thai 2012; 96.
15. Turajane T, Thitiset T, Honsawek S, Chaweewannakorn U, Aojanepong J, Papadopoulos KI. Assessment of chrondrogenic differentiation potential of autologous activated peripheral blood stem cells on human early osteoarthritic cancellous tibial bone scaffold. Musculoskelet 2014; 98: 35-43.
16. Lee H, Park KM, Joung YK, Park KD, Do SH. Platelet-rich plasma loaded hydrogel scaffold enhances chondrogenic differentiation and maturation with up-regulation of CB1 and CB2. J Control Release 2012; 159: 332-7
17. Sundman EA, Cole BJ, Karas V, Valle CD, Tetreault MW, Mohammed HO, et al. The anti-inflammatory and matrix restorative

mechanisms of platelet-rich plasma in osteoarthritis. Am J Sports Med 2014; 42: 35-41.
18. Filardo G, Kon E, Buda R, Timoncini A, Martino AD, Cenacchi A et al. Platelet-rich plasma intra-articular knee injections for the treatment of degenerative cartilage lesions and osteoarthritis. Knee Surg Sports Traumatol Arthrosc 2011; 19: 528-35.
19. Vilchez-Cavazos F, Millan-Alanis JM, Blazquez-Saldana J, Alvarez-Villalobos NA, Pena-Martinez VM, Acosta-Olivo CA, et al. Comparison of the clinical effectiveness of single versus multiple injections of platelet-rich plasma in the treatment of knee osteoarthritis. Ortho J Sports Med 2019; 7: 2325967119887116.
20. Laver L, Maron N, Dnyanesh L, Mei-Dan O, Espregueira-Mendes J, Gobbi A. PRP for degenerative cartilage disease: a systematic review of clinical studies. Cartilage 2017; 4: 341-64.
21. Dhurat R, Sukesh M. Principles and methods of preparation of platelet-rich plasma: a review and author's perspective. J Cutan Aesthet Surg 2014; 7: 189-97.
22. Vannabouathong C, Bhandari M, Bedi A, Khanna V, Yung P, Shetty V et al. Non-operative treatments for knee osteoarthritis: an evaluation of treatment characteristics and the intra-articular placebo effect. J Bone Joint Surg 2018; 7: 1-11.