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Moving toward targeting the right phenotype with the right platelet-rich plasma (PRP) formulation for knee osteoarthritis

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***Abstract*:** Intra-articular injections of platelet-rich plasma (PRP) and other novel blood-derived products developed specifically for osteoarthritis (OA) can provide pain relief and potential benefits in disease progression. Meta-analyses show the clinical superiority of PRP compared with other intra-articular injections, but results are modest and the effect sizes are small. PRP injections in knee OA are performed indiscriminately, but the clinical response varies enormously between patients because of an array of mixed OA phenotypes. Subgroup analyses are scarce; some studies stratify patients according to radiographic severity and found better results in early OA, without consensus for more advanced stages of the condition. Parallel identification of soluble and imaging biomarkers is essential to personalise and leverage PRP therapies. The inflammatory phenotype is most interesting from the PRP perspective because PRPs modulate inflammation by releasing a large pool of chemokines and cytokines, which interact with synovial fibroblasts and macrophages; in addition, they can modulate the innate immune response. No soluble biomarkers have been discovered that have implications for OA research and PRP interventions. Clinical examination of patients based on their inflammatory phenotype and imaging identification of pain sources and structural alterations could help discern who will respond to PRP. Synovial inflammation and bone marrow lesions are sources of pain, and intra-articular injections of PRP combined with subchondral bone injection can enhance clinical outcomes. Further refining ultrasound phenotypes may aid in personalising PRP therapies. Intra-articular delivery combined with injections in altered ligamentous structures, medial and coronal ligaments or premeniscal pes anserinus showed positive clinical outcomes. Although the evidence supporting these approaches are weak, they merit further consideration to refine PRP protocols and target the right OA phenotypes.

Keywords: chemokines, growth factors, intraarticular therapy, Knee osteoarthritis, phenotype, platelet-rich plasma, synovium

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Introduction

Osteoarthritis (OA) is a chronic debilitating joint disease: its impact in the knee can be devastating.[1,2] The prevalence of OA has doubled when comparing people living during the early industrial (1800–1900) and the modern postindustrial eras, even after controlling for obesity and age.[3] This escalation is not fully understood, but current hypotheses suggest that the underlying reason could be an evolutionary mismatch between our ancestral genes, developed to meet the needs of active hunters with a high fibre diet, and modern environmental challenges, that is, sedentarity, obesity, diet and ageing.[4]

OA is progressive and, at the end stage, patients are severely limited by chronic pain and disability, when total knee replacement (TKR) is the only alternative, with a huge rise in its burden worldwide. For example, the projected growth of TKR in Australia is expected to be 276% by 2030, with healthcare costs of $AUD 5.32 billion.[5] In a different time frame, from 2012 to 2050, the rise of TKR in the United States (US) is expected to be 855% with over half of the recipients aged below 65.[6,7]

Given these data, the need for enhanced non-surgical therapies, not only palliative, but to manage early stages and prevent OA progression, is apparent. Analgesics, including paracetamol, topical and oral non-steroidal anti-inflammatory drugs (NSAIDs) and opioids, remain the basis of pharmacological treatment. Intra-articular delivery of corticosteroids can provide short-lived improvement without systemic exposure,[8–10] and is commonly prescribed in general practice, even though the benefits of physical therapy are superior at 1 year.[11] Intra-articular injections of platelet rich plasma (PRP) can provide symptomatic pain relief and potential benefits in the modulation of inflammation.[12,13] Furthermore, novel blood-derived products specific for OA are emerging.[14,15]

However, clinical response varies enormously between patients, probably because OA involves different etiologies and pathogenic mechanisms, and thus an array of mixed phenotypes. Which patients are the best candidates for blood-derived therapies thus remains to be elucidated.[16,17]

In this forward-looking perspective, we provide a brief overview of PRPs and other blood-derived products in current clinical use. Secondly, we review the recent literature on the role of PRPs in modulating inflammation and the innate immune response in the knee. Finally, as there is interplay between mechanical damage and inflammation in OA, we will discuss briefly how recent work performed in OA phenotyping based on ultrasound imaging and soluble biomarkers might impact current PRP research.

The blood-derived product family

Many commercially available blood-derived products have been introduced in clinical practice and are being investigated clinically. PRP preparations are used in different clinical areas, including dentistry, musculoskeletal medicine, plastic surgery, *in vitro* fertilisation and wound management.[18–21]

PRPs are autologous or allogeneic blood derivatives. The latter have shown low immunogenicity as confirmed in a recent randomised controlled study, in which allogeneic PRP was used as an adjuvant in the management of chronic wounds.[22] The clinical safety of allogeneic PRP was also corroborated in a randomised controlled trial encompassing 75 patients injected with allogeneic PRP in plantar fasciitis.[23]

PRPs contain a supraphysiological concentration of platelets, and optionally leukocytes, in a limited volume of plasma.[24] The therapeutic use of PRP is based on platelet biology. Platelets are non-nucleated cells containing different types of storage granules with a broad array of molecules involved in tissue healing. Novel molecular findings are expanding the number of proteins, and a recent proteomic study identified 125 proteins associated with tissue healing.[25] Moreover, the traditional family of PRPs includes different formulations that can differ in platelet and leukocyte content as well as activation system. A second generation of protocols/products, such as hyperacute autologous serum (HAS)[14,26] (hypACT™ inject, Orthosera, Krems, Austria), gold-induced, autologous-conditioned serum (Goldic®, Arthrogen GmbH, Gmund am Tegernsee, Germany),[27] or autologous cytokine rich serum (ACRS) (Qrem™, Barcelona, Spain), still contain the platelet secretome, but fibrin and other insoluble proteins formed upon coagulation have been removed.[28,29] Whether to include fibrin(ogen) when injecting a joint is controversial; fibrin could be a useful vehicle to allow longer times for the product to remain within a joint as it facilitates a slow delivery of cytokines.[30] However, the rate of clearance of intra-articular therapies from the synovial fluid by lymphatic drainage is not size selective (in the range of molecular radius between 2 nm and 10 nm). Nevertheless, cross-linked molecules of high-molecular weight hyaluronan appear to encounter steric hindrance en route to the terminal lymphatics and could be cleared more slowly. Indeed, single injection of hyaluronan has been developed in part based on this assumption.[31,32]

PRPs are safe,[12,18] but most therapeutic applications, including OA management, are considered off-label. This fact contrasts with the newly developed protocols relying on blood-derivatives, which are based on a candidate molecule approach and commercialised exclusively for OA management through investigational new drug (IND) approval. Novel protocols involve the selection of specific blood components and precise molecular targets. For example, autologous alpha-2 macroglobulin (A2M) can be prepared from peripheral blood through multiple centrifugations and filtration using a commercialised protocol

(APIC™, Cytonics, Jupiter, FL, USA). A2M is an endogenous regulator of catabolic cytokines and inhibitor of serine proteases and MMPs activities. Accordingly, A2M injections were chondroprotective in experimental post-traumatic OA.[33] Moreover, post-marketing pragmatic data revealed moderate clinical benefits in patients with knee OA after a single injection.[34] The results of a controlled clinical trial [ClinicalTrials.gov identifier: NCT03656575] comparing the levels of inflammatory cytokines in the synovial fluid of patients treated with intra-articular injections of A2M, corticosteroids or PRP, are expected soon.

Another illustrative example of the candidate molecule approach is the so-called autologous protein solution[35,36] (APS, nSTRIDE® APS Kit, Zimmer Biomet). This protocol processes 60 ml of peripheral blood and concentrates inhibitors of inflammatory cytokines, i.e. interleukin 1 beta (IL-1b) and tumour necrosis alpha (TNF-a), in particular the endogenous receptor antagonist (IL1Ra) and soluble receptor (sTNFR).[37] Blocking these cytokines is relevant, as they are main effectors of joint catabolism. Likewise, Orthokine® uses a special syringe containing CrSO4-treated grade glass beads in order to promote IL-1Ra synthesis and accumulation (currently marketed as Orthogen® AG, Düsseldorf, Germany).[15,38]

Mechanism of action of PRP, with a focus on inflammation

Platelets are released from the bone marrow in the bloodstream, where they circulate for 7–10 days, as fragments of their precursor cell, the megakaryocyte. In the body, platelets interact with leukocytes and endothelial cells under shear forces. Much of the understanding of the role and function of PRP examines the interactions of platelets outside the bloodstream, with joint cells, i.e. chondrocytes, synovial fibroblasts and macrophages. Following activation, platelets aggregate and release by exocytosis their granule content, which is the platelet secretome. The latter consist of small molecules from dense granules and a large pool of cytokines, chemokines and growth factors from the alpha granules, which are involved in tissue repair. Although attention has been focussed on mitotic and anabolic actions of growth factors, such as transforming growth factor beta1 (TGF-b1), platelet derived growth factor (PDGF) and insulin growth factor (IGF-1), we and others emphasise the implications of immunomodulatory cytokines released from platelets,[13,39] including regulated on activation normal T cell expressed and secreted (RANTES/CCL5), platelet factor (PF-4/CXCL4), epithelial neutrophil-activating peptide 78 (ENA-78/CXCL5) and neutrophil activating peptide 2 (NAP-2/CXCL7). In addition, receptor activator of nuclear factor kappa B ligand (RANKL) and P-selectin are involved in the interactions of platelets with other cells, mainly innate immune cells. Platelets present a large variety of membrane receptors.[40,41]

PRP therapies for OA have been developed based on several assumptions beyond tissue homeostasis: (1) anti-inflammatory effects of chemokines, and subsequent anti-catabolic effects; (2) immune modulation and (3) anabolic actions of growth factors. However, the number of platelets, for unit of volume, is not necessarily associated with clinical success in the conditions in which PRP are used. Platelets contain proteins with opposing actions; hence, increasing platelet number does not modify this balance. Considering this fact, PRPs could be considered as a buffered molecular system. Also, some molecules have pleiotropic functions, i.e. their signalling pathways and biological consequences depend on their concentration or tissue context. A relevant example is TGF-β, which can counteract pathological changes in a young healthy joint while it is a driving force of pathology in OA joints during ageing.[42]

Synovial cells are the primary targets of intra-articular injections of PRP because they contribute greatly to the molecular content of synovial fluid that bathes the whole joint. When an inflammatory state is present, synovial cells synthesise TNF-α and IL-1β that stimulate the synthesis of metalloproteinases (MMPs) whose enzymatic activities lead to cartilage breakdown. Regarding the interactions of PRP with synovial cells, two facts were established in the earlier research. Exposure of synovial fibroblasts, from patients with established OA, to PRP releasate induced the synthesis of hyaluronic acid (HA) and hepatocyte growth factor (HGF).[43] The former involves enhancement of lubrication while HGF downregulates chondrocyte inflammation through interaction with toll-like receptors and subsequent modification of nuclear factor kappa beta (NF-kB) signaling.[13,44] HGF is also involved in stem cell activation following injury.[45] PRP decreased joint catabolism through downregulation of MMP-13 in OA synoviocytes co-cultured with chondrocytes.[46] Moreover, PRP can down regulate

TNF-α, interleukin 6 (IL-6) and IL-1β production by suppressing serine/threonine kinase 1 (AKT1), phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) and NF-kB (PIK/AKT signalling) in lipopolysaccharide (LPS)-exposed rheumatoid fibroblasts like synoviocytes.[47] Yet, PRP promoted rheumatoid arthritis fibroblast-like (RA-FSL) cell migration, invasion and adhesion through cytoskeletal reorganisation and upregulation of MMP-1.[48] Despite experimental contradictions, three PRP injections 1 week apart were safe and effective in RA patients.[49] Further investigations are required.

Not only synovial fibroblasts but also resident macrophages (i.e. synoviocytes type A), which cohabit with synovial fibroblasts and modulate their activities, can be triggered by the secretome of PRP. Current data assign predominant roles to tissue macrophages in failed resolution of inflammation and loss of joint homeostasis.[50] Macrophage dysfunction in knee OA has clinical implications,[51] and synovial fluid biomarkers reflecting macrophage activities allow patients with an inflammatory endotype to be identified. The panel of biomarkers composed by vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule 1 (ICAM-1), tissue inhibitor of metalloproteinases (TIMP-1), vascular endothelial growth factor (VEGF), MMP-3 and monocyte chemotactic protein (MCP-1) is associated not only with OA symptoms, but also with macrophage infiltration and with soluble forms of macrophage cell surface markers, e.g. CD14 and CD163. Also, all these biomarkers, except MCP-1, correlated with neutrophil elastase; high levels of elastase in the synovial fluid can predict knee OA progression. However, the relevance of PRP injection in macrophage changes is limited by our current understanding of the intermediary states of macrophage polarisation (between classically activated M1 and alternatively activated M2).[52]

On the other hand, platelets can attenuate the inflammatory response by acting on the innate immune system. This property stems from platelet characteristics, i.e. their ability to secrete diverse pro- and anti-inflammatory signals to initiate and modulate immune mechanisms. These include immunosuppressant such as TGF-β, abundant chemokines including PF4, NAP-2, RANTES and MIP-1a. Moreover, CD40L allows platelet interaction with other cells from the immune system, and platelets regulate neutrophil degranulation and can stimulate neutrophil phagocytosis. Thrombocytopenia reduces leukocyte infiltration in various models of acute and chronic inflammation.[53] Production and release of cytokines by monocytes can also be regulated by platelets. For example, thrombin activated platelets induce the expression and secretion of MCP1 and IL8 by monocytes in a P-selectin-dependent manner. In addition, molecules released from dense granules, such as serotonin and histamine, can increase the permeability of the synovium.

Badsha *et al.* recently revealed proof of concept of the immunomodulatory effects of PRP in four RA patients[54]; ultrasound evidenced clinical improvement and amelioration of joint inflammation. A placebo-controlled trial in OA patients revealed a reduction in plasma concentrations of inflammatory and proangiogenic factors (investigative biomarkers) compared with controls.[55]

Non-canonical PRP mechanisms have been described in haemophilia patients, in whom repeated bleeding and haemarthrosis cause chronic synovitis and arthropathy. PRP interfered with the conversion of haemoglobin to methaemoglobin and was effective in patients with chronic haemophilic synovitis pain and bleeding episodes.[56]

However, the actual mechanism of the beneficial action of PRPs in OA remains elusive.

How work performed in OA phenotyping might impact PRP therapies and research

Recent meta-analyses have shown some superiority of PRP compared with other intra-articular injections,[19,57] but results are modest and the effect sizes, i.e. the degree to which PRP influences pain or function at a given time, are small. This is likely a consequence of the failure to identify *a priori* in which patients PRP works and with which protocol.[16,17]

Traditionally, patients have been stratified according to radiographic severity, i.e. assessing structural damage, in terms of joint space narrowing and osteophyte formation, using the Kellgren–Lawrence (KL) or Ahlbäck scores. In this context, good subgroup analyses are scarce but, according to a recent consensus based on Delphi methodology, there was strong agreement (77.5%) supporting PRP injections in early knee OA (KL grade II), without consensus for the other stages of the condition.[55] Better results with PRP are obtained in patients who are male, young, with a lower degree of OA and low body mass index (BMI).[58] One reason might be the differences in PRP composition, in terms of inflammatory cytokines (IL-1β and TNF-α) and anabolic growth factors (TGF-β and IGF-I) between young healthy donors and older males with knee OA.[59] Also, there could be differences in cartilage biology, as these changes were associated to decreased anabolic response (i.e. COL2a1 and Sox 9 gene expression) in chondrocytes from older patients and upregulation of inflammatory molecules in macrophages, i.e. TNF-α and MMP-9.[59] Nevertheless, PRP injections exerted an analgesic effect comparable with corticosteroids in old patients with severe disease.[60]

In the end, intra-articular injections of PRP are administered mostly indiscriminately. Even though PRP therapies could benefit enormously from trial enrichment markers, in the early studies there were no major biomarker discoveries that had implications for OA research and PRP interventions.[61]

Biomarker research in PRP science is scarce, but inflammation is implicated. In patients with moderate knee OA combined with suprapatellar bursitis, proteomic analyses of the synovial fluid indicated a decrease in proteins associated with inflammation and an increase in proteins associated with chelation and anti-aging.[62] However, clinical qualification was not performed, and no biomarker was linked with clinical end-points. Other authors showed a reduction in cartilage catabolism after PRP, through serum peptide of the alpha-helical region of type II collagen.[63] Intra-articular PRP improved the appearance of inflamed synovium, and ultrasound changes (reduced vascularity and less effusion) correlated with clinical outcomes.[64] Calcitonin gene-related peptide expression mediates pain reduction and down-regulation of synovial inflammation induced by PRP.[65]

Advanced biomarkers, both anatomic and soluble biomarkers, reflecting several pathophysiological mechanisms, have been researched extensively,[66,67] mainly in the Osteoarthritis Research Society International (OARSI) framework, to improve OA management.[68] Accordingly, OA phenotypes were defined as 'subtypes of OA that share distinct underlying pathobiological and pain mechanisms and different course of the disease', merging in similar structural changes in final stages.

Higher degrees of synovitis and bone marrow lesions (BMLs) are associated with more severe pain. Intra-articular injections could down-regulate synovial inflammation but cannot reach the altered subchondral bone. In this context, Sánchez *et al.* proposed the combination of intraosseous and intra-articular injections and assessed clinical changes at 6 and 12 months in a controlled study involving patients with BMLs in severe OA.[69] The minimally clinical important improvement was higher in patients that had one intraosseous injection in addition to the intra-articular injection. Similar results were revealed in subsequent clinical studies.[70,71] These studies are, however, highly heterogeneous with a high risk of bias, and intraosseous injections cannot yet be recommended. Nevertheless, an optimal therapy should target the predominantly altered tissue and/or source of pain.

Following this paradigm, Sit *et al.* proposed to inject PRP not only intra-articularly,[72] but also to apply PRP on extrasynovial ligaments, as alterations in the medial collateral ligament and medial coronary ligament can be involved in OA.[73] Grounded on these concepts, 3 ml of (PRP + 1% xylocaine) (6:1, vol:vol) were injected intra-articularly, 2 ml over the medial coronary ligament and 2 ml over the proximal medial collateral ligament (MCL) with positive clinical results. However, although improvements in pain (ICOAP) and WOMAC (all dimensions) were significant, the small number of patients and the absence of a control group limit reliability. Similarly, intra-articular PRP injections (4 ml) associated with premeniscal peranserinus injections (2 ml) induced positive proteomic changes in the synovial fluid along with pain reduction and functional improvement, and were proposed as a treatment option for elderly patients.[74]

Administration of PRP under ultrasound guidance permits the right intra- or extra-articular targets, often the source of pain, to be identified and increases the likelihood of response. Moreover, ultrasonography can help to identify patient subgroups according to imaging biomarkers, and help to qualify outcome assessments.[75,76]

Key needs in PRP therapies

The marketed clinical efficacy for intra-articular PRP injections is 78% (49–97%),[77] but counselling in favour or against PRP therapies is challenging. Intra-articular PRP treatment is considered off-label, thus insurance companies and public health systems do not provide coverage. PRP injections cost about US $714 for one knee,[77] and they are not considered cost-effective because they do not delay TKR.[78] In fact, they do not influence substantially structural and clinical endpoints, a failure attributed to extreme variability of PRP products and intervention protocols, i.e. number of injections, volume, ultrasound/imaging guidance and injection technique.

The major challenges are to improve our understanding of the mechanism of action of PRP in the joint to optimise PRP formulations, identify soluble biomarkers and clarify inflammatory aspects of OA pathophysiology; in parallel, we must improve imaging interventions based on investigative ultrasound biomarkers, such as synovial hypertrophy, joint effusion and involvement of tendons and ligaments.[79] Moreover, to capture relevant changes, early or intermediate outcome measurements should be tailored to the clinical phenotypes of OA.[80]

Conflict of interest statement

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