Regenerative Medicine in Orthopedics: What do we Know, What do we not Know, What Should we Know?

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Learner Objectives:
-Define and discuss the differences between various platelet rich plasma and stem cell preparations
-Discuss the evidence for outcomes after regenerative medicine treatments in dogs
-List indications and applications for regenerative medicine options after injury

Orthobiologics also referred to, as regenerative medicine is a new emerging modality for a variety of disease problems. In general, the goal of regenerative medicine is to take a solution with a high concentration of growth factors and anti-inflammatories to an area of otherwise poor healing. To get the best response from regenerative medicine one must have a definitive diagnosis and must be able to treat or manage the underlying condition. It's designed to be another "tool in the tool-belt" for the multimodal management of musculoskeletal diseases.

Platelet Rich Plasma (PRP):
PRP is blood plasma concentrated with platelets (PLT) designed for injection for musculoskeletal injuries. Within these platelets there are large reservoirs of alpha granules that release bioactive proteins and growth factors. These proteins have been shown to initiate and/or accelerate tendon, ligament, and cartilage repair. The alpha granules within platelets are a contain a rich source of growth factors such as transforming growth factor β (TGF-β), platelet derived growth factor (PDGF), insulin like growth factor (IGF-1), vascular endothelial growth factor (VEGF), epidermal growth factor (EGF), and fibroblast growth factor (FGF).

The generalized protocol for PRP collection begins with collecting 10-60 ml of blood from the patient, then centrifuging the blood or filtrating it to produce the PRP. For OA PRP is typically injected via blind intra-articular, fluoroscopic or digital radiography guided. For soft tissue injuries the PRP should be injected into the lesion via ultrasound guidance not just into the local area. The diagnosis of "soft tissue injury" is now obsolete, as we should try to determine the exact tissue that is damaged.

What should also be taken into consideration is the system being using to produce the PRP. Systems work by either gravity spin or spin separation. In addition, the system should have a good price point and value. However, the absolute most important consideration is that the system has been shown to be repeatable for the species that it is being used on.

Ideally in any product we want the PLT concentration to increase 6-7 fold (too high such as greater than 10-12 fold can actually cause issues such as a joint flare).

Given that PRP is a relatively new technology there are several questions that need to be addressed:
How many injections should one receive?
-In a general sense over 50% of patients need more than one injection separated by about 2 weeks. However, if no effect is seen after 3 injections, it is likely not to be helpful.

Do all disease conditions and severities need the same PRP solution?
-Originally, it was thought that all disease conditions and all disease severities should be treated with the exact same PRP solution. This is likely not true as a patient with mild OA may need something different in the PRP versus a patient with severe OA. Further expanding on this it is possible that a patient with a grade 2 supraspinatus tendinopathy would need a differing solution of PRP from a patient with moderate OA.

Another big question is what exactly should be in the PRP product: platelets alone, platelets with white blood cells (WBCs), and/or platelets with red blood cells (RBCs), and how do we process the PRP in a repeatable fashion?

In terms of separation we want to spin the blood to allow gravity to separate the cells based on weight. Currently, the thoughts in a post spin PRP product are an increased concentration of platelets that have primarily alpha granules with plenty of growth factors. Monocytes are considered acceptable in that they are associated with an increase in cellular metabolism and collagen production in fibroblasts. Also, we want a decrease in the release of anti-angiogenic cytokines, which makes sense in that one of the properties of PRP, is angiogenesis. Currently the thought on lymphocytes in the PRP is unknown. PLT's have been shown to activate peripheral blood mononuclear cells, which when mediated by an increase in IL-6 expression can help stimulate collagen production.

What may be considered to be harmful or not needed in the post spin PRP solution are RBC's which have been shown to cause direct damage to the cartilage and synovium via iron-catalyzed formation of ROS when injected intra-articular. We likely don't want the PRP to contain any WBC's that cause an inflammatory reaction, as inflammatory mediators have been shown to cause synoviocyte death. Neutrophils are probably the biggest concern in that they increase the concentration of inflammatory mediators in the area. While these considerations are more relevant to OA management, the solution for non-operative soft tissue orthopedic injuries may be different. Many of the chronic soft tissue injuries are repetitive in nature so there is replacement of normal tissue architecture with fibrous tissue. It may very well be that in some of these chronic injuries that a certain amount of WBCs helpful. This would be considered a leukocyte-rich PRP versus the more commonly used leukocyte-poor PRP.

Thankfully, work has been done to characterize PRP solutions made by different manufacturers. One of the first studies in veterinary medicine that looked at the processing of PRP was published by Stief, et al. in VCOT in 2011, which found that canine ACP processed using the manufacturer's recommendations did not show the same specifications as what was reported in human ACP using the same guidelines. In 2015, Carr, et al evaluated 5 commercially available PRP products. The goal of this study was

to utilize a multicenter approach to determine the repeatability when following manufacturer's recommendations. This study ran pre-spin CBC's on all samples, processed the sample according to the manufacturer and then ran post-spin CBC's looking at platelet (PLT) concentration, white blood cell concentration (WBC), and red blood cell (RBC) concentration. Also, in 2015 Franklin, et al. completed a similar study with 5 commercially available products using similar (not exact) methodology. Looking at these studies from a broad sense: every PRP machine produces a similar solution within its self; however, there is great variability between machines in terms of the solution produced. Unfortunately, while these studies prove validation for various products they do not claim and efficacy.

From a PRP product standpoint I suspect in the future, we may actually want a customizable product for patient specific management In, other words the ideal system will allow the user to determine the concentration of PLT's, RBC's, and WBC's for the disease condition they are managing.

There is a multitude of literature available for PRP. A quick PubMed search reveals over 10,000 peer reviewed articles. However, clinical evidence using objective outcome measures with appropriate follow up for PRP alone for veterinary patients remains poor. Currently, there are only 3 canine studies for OA showing some positive effect with intra-articular injections; however, the effects were short lived. There is 1 study looking at canine tendinopathy, thus the data is weak. In addition, there is 1 study looking at bone healing compared to a control that found no improvement in osseous union with the addition of PRP. Lastly, one study revealed that injection of PRP into the contralateral stifle at the time of TPLO did not change the percentage or timeframe for contralateral cruciate rupture.

Mesenchymal Stem Cell (MSC):
For this discussion stem cells are referred to as adult derived undifferentiated mesenchymal stem cells (MSC). MSC are said to be multipotent in that they can give rise to multiple but limited number of lineages. Much remains to be learned about stem cells; in clinical practice there are two main sources: adipose derived (AD-MSC) and bone marrow derived (BM-MSC). Within both AD-MSC and BM-MSC there can be culture expanded techniques or in-house preparations such as the bone marrow aspirate concentrate (BMAC) if bone marrow is used or the stromal vascular fraction (SVF) if adipose tissue is used.

To identify MSC, cells must have 3 specific characteristics:
1) Adherence to plastic culture dishes and form fibroblast like colonies (CFU).
2) Capacity to differentiate into various specialized cell lineages. MSC have been induced into adipogenic, chondrogenic, osteogenic, myogenic, and neurogenic like lineages among others.
3) Expression of defined cell surface marker profiles using immunohistochemistry and flow cytometry.

Unfortunately, what makes identification even more challenging is that some labs will use differing cell surface marker to identify a stem cell. This makes extrapolating data from various studies challenging if not impossible.

Some stem cell biologist would also suggest a fourth criteria; there has to be documentation of tri-lineage differentiation of the cells in question to say if the cell is a MSC or not. The problem with this approach is that it is not logistically or financially feasible to process stem cells in-clinic for clinical use. Therefore, technically to use "stem cell therapy" the adipose tissue or bone marrow would need to be sent to an outside lab for culture expansion following the 3 or 4 criteria listed above. To avoid confusion and not misleading the general public BMAC and SVF SHOULD NOT be considered "stem cell therapy". Both BMAC and SVF are heterogeneous solutions that contain small portions of MSC (BMAC produces about 0.02% of CFU and adipose 3-5% CFU; personal communication with Dr. Brian Saunders). Unfortunately, in human medicine the FDA prohibits any manipulation of cells as seen with culture expanded or allogeneic use. In the coming years we may see these same restrictions applied to veterinary medicine leaving us only with the usage of BMAC or SVF.

There is controversy behind using MSC, mainly because of the public's perception of what it is and where it is coming form. The problem much like with PRP is the lack of standardization makes treating individuals with the exact same product is difficult. The original thought of MSC was simply just to replace lost tissue and the stem cells would regenerate new tissue. However, research has shown that the #1 mechanism of repair is the release of trophic factors. These trophic factors in the form of cytokines and chemokines release different growth factors and also provide an anti-inflammatory environment as well as help with immune system modulation.

Autologous stem cell therapy is essentially harvesting the source of the tissue, isolating and expanding the MSC then returning it back to the same patient. Bone marrow derived stem cells actually contain both hematopoietic stem cells and MSC. The two ways to collect MSC from bone marrow are culture expanded and bone marrow aspirate concentrate (BMAC). Adipose derived stem cells contain MSC, and the two ways to collect MSC from fat are culture expanded and stromal vascular fraction (SVF). Currently, at this time there is still no true superior source of MSC, so please don't let a company give you false impressions when evaluating their product.

Evidence base for stem cell usage much like PRP is lacking for clinical evidence using objective outcome measures with appropriate follow up for stem cells alone for veterinary patients. While PubMed will reveal over 375,000 peer-reviewed articles for stem cells, the author only considers there to be 5 studies for OA management, and 1 case report for canine tendinopathy. Four of the 5 studies showed some positive improvement for OA; however, the duration was short lasting only 3-6 months. The case report for canine tendinopathy revealed a positive outcome with orthotic support.

There is some evidence to suggest that combination therapy; using both PRP and BMAC/SVF may have a better outcome over using just one alone. However, veterinary

studies using combination therapy are retrospective and don't include a control group making the scientific evidence weak to support its usage over either one alone.

Stem cell therapy has been used for OA management more commonly as well as various tendinopathy conditions, partial cranial cruciate ligament tears, and fractures. I would urge extreme caution considering stem cell therapy for partial CCL tears; given that CCL pathology in the canine is due to a degenerative process, not a traumatic one.

Post injection management protocols for regenerative are largely unknown but commonly include some form of formal rehabilitation therapy. Therefore, the severity of the condition being managed, the duration of the injury, and the overall condition of the patient should be taken into consideration. Immediately following regenerative medicine pROM, stretching, and manual therpaies should begin as well as slow walking on leash for elimination. Around the 10-14 day mark isometric therapeutic exercises are started as well as manual therapies and modalities continued until the patient can progress into eccentric/concentric muscle building.

It should be noted that we want to try and avoid the use of NSAIDs or steroids for 1-week prior and 2 weeks post injection (up to 4-6 weeks for MSC therapy). This decision is based on human studies; however, recent work has called this into question. Furthermore, ice packs/cryotherapy should be avoided for the first 2 weeks as it has been shown to decrease platelet activation. Additional unknowns are how certain rehabilitation modalities such as shockwave, laser, and therapeutic ultrasound affect regenerative medicine. Until further research is completed shockwave therapy should be used with caution (use before, or wait until at least 60 days following). If therapeutic ultrasound is used, a pulsed mode is better than continuous. For laser therapy class IIIb may be good and actually help activate the cells; however, the intensity of class IV may be too much.

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