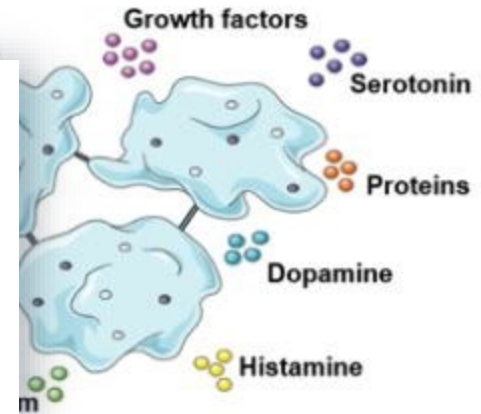


Platelet Lysate

Potential Clinical Advantages and
How to Prepare



Financial Disclosures:

None



Who I am

John Knab, MD

Medical Director

Center for Pain Management

Wilmington, NC



Learning Objectives:

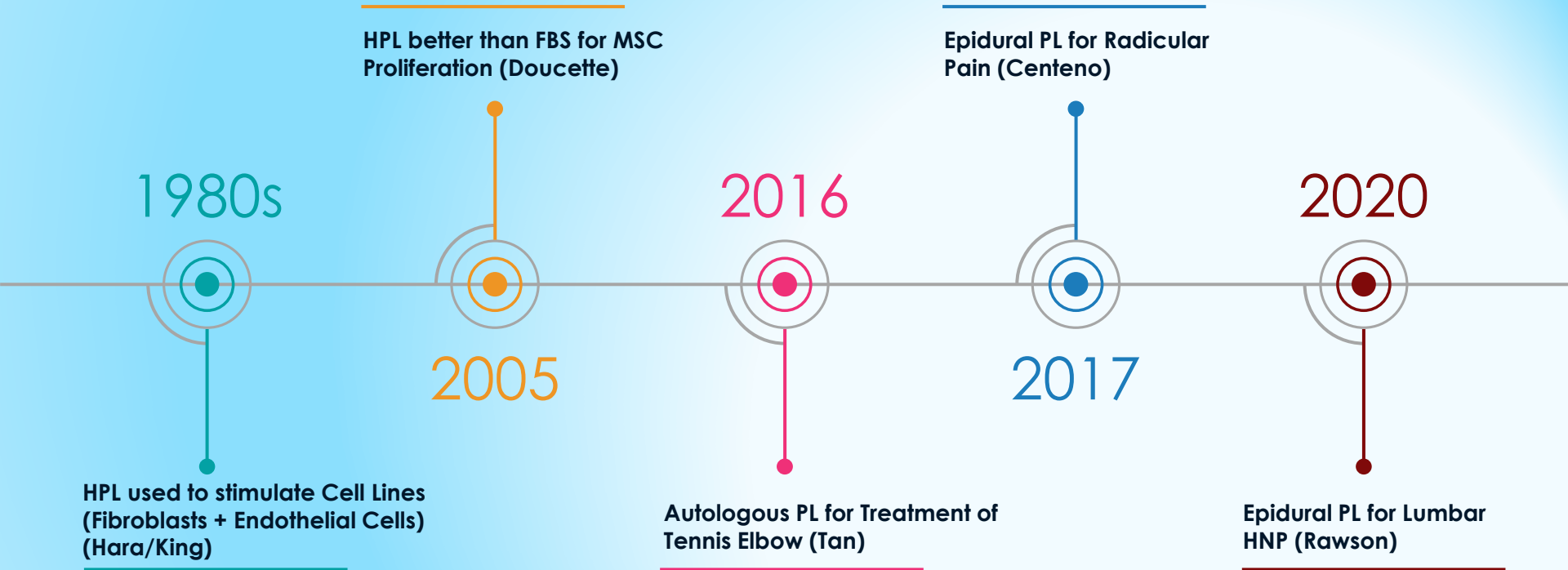
1. History + Why?
2. Releasate vs. Lysate?
3. How to Make It
4. Clinical Applications

1

Platelet Lysate

History

Platelet Lysate: Historical Timeline

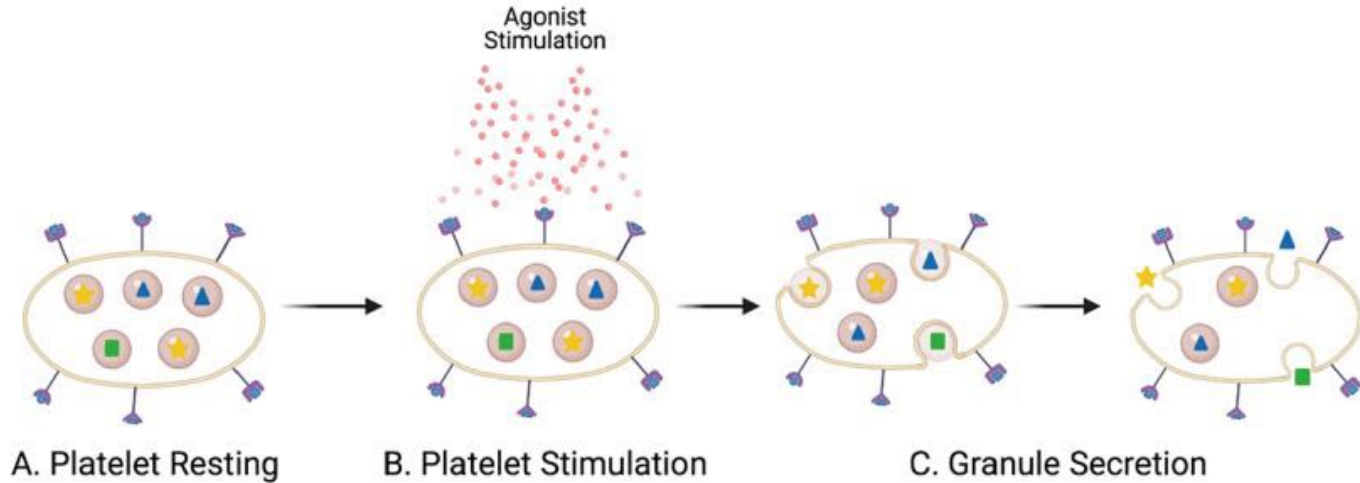


2

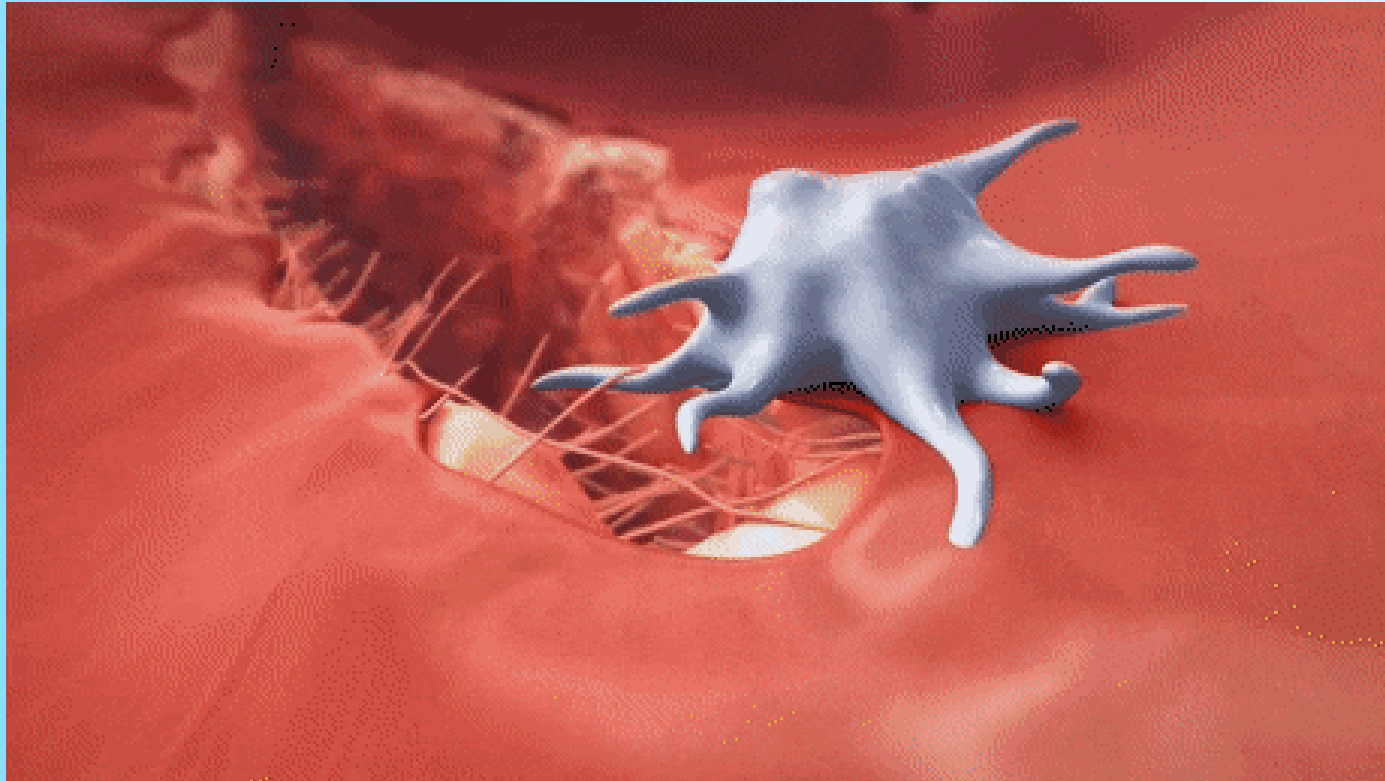
Platelet Lysate in Orthobiologics:

When and Why?

Platelet Therapy in MSK medicine: PRP



Platelet Degranulation

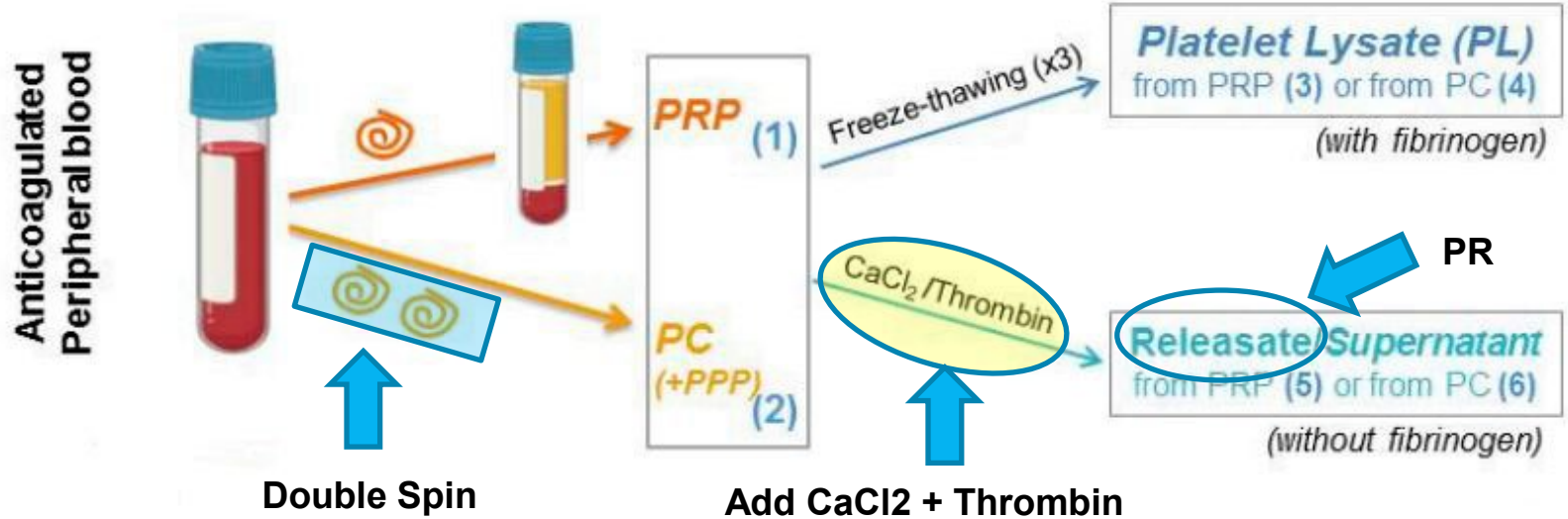


3

Lysate vs Releasate What's the Difference?

Definition + Common Preparation Techniques

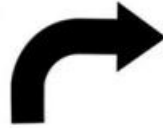
Platelet Release



Platelet Lysate: Mechanical Rupture

(a)

Lysis of
Platelet



Lysis of
Platelet
Content



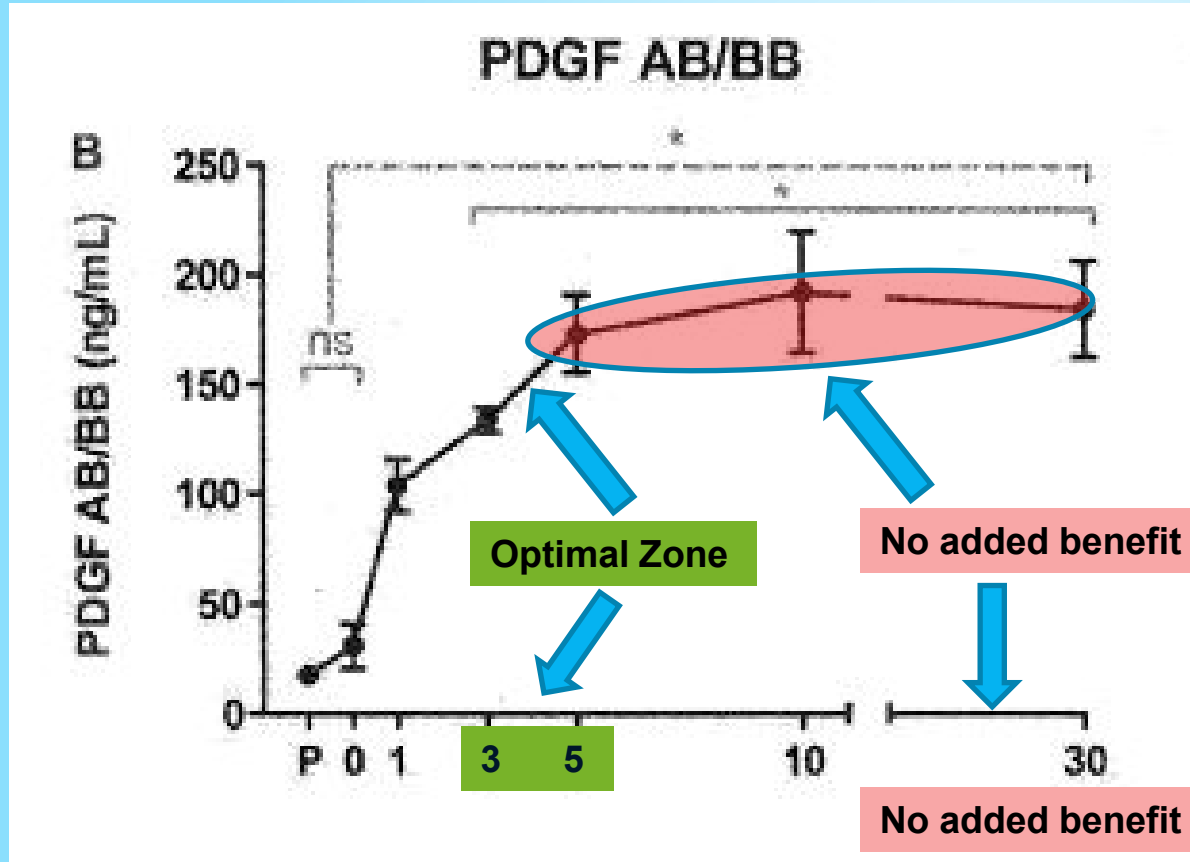
-  α - Granules
-  δ - Dense Granules
-  Lysosomes

> Chemostatic Factors
> Angiogenic Factors
> Growth Factors
> Proteases
> Cytokines

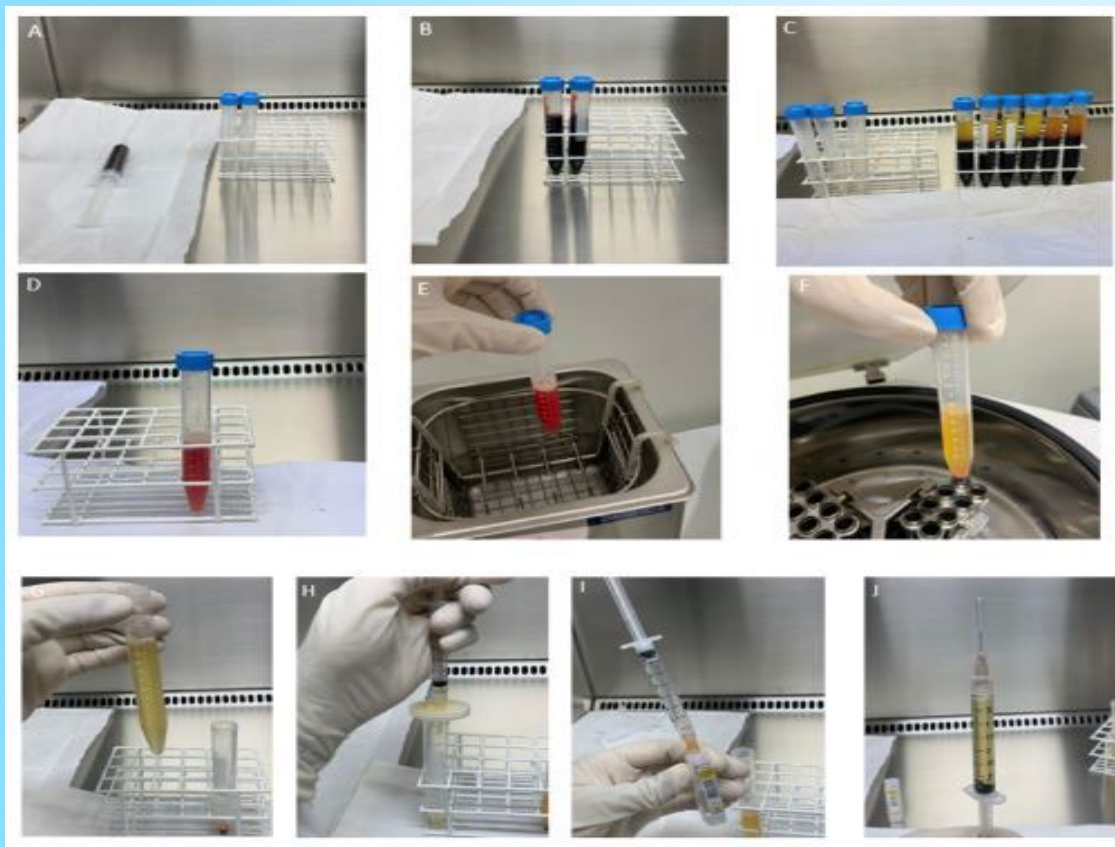
Release of
Platelet
Content



Freeze/Thaw – How Many Cycles?



Ultrasonication @ > 20 KHz x 30 min



Bernardi et al
Cytotherapy
2013

Da Fonseca et al
J Clin Orth
Traum 2021

Platelet Lysate – How We Make It



Platelet Lysate – Freeze/Thaw x 3



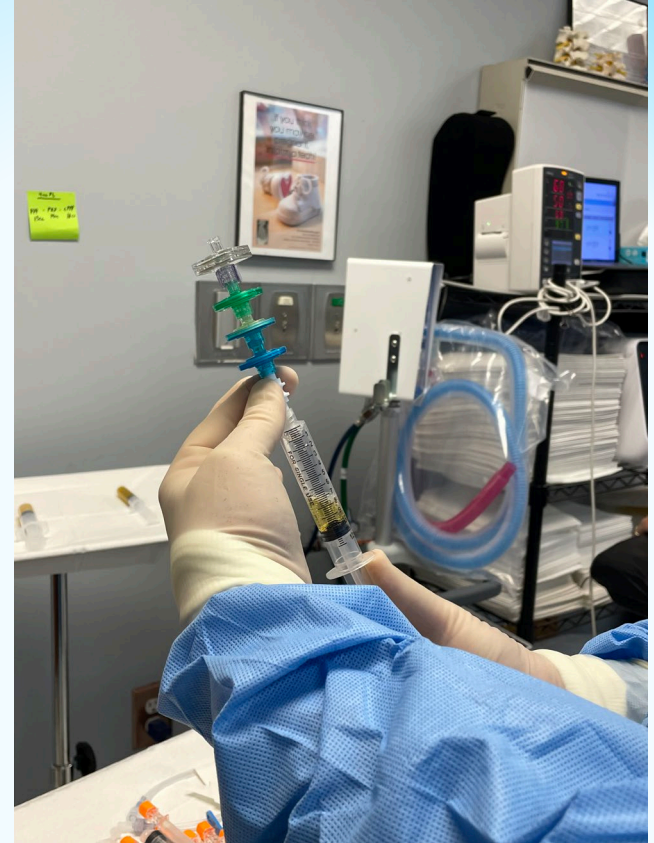
-80°C \leftrightarrow 52°C



x3 Cycles



Platelet Lysate – Filtration



4

Platelet Lysate Clinical Use

Published Data + Clinical Experiences

Platelet Lysate GF Levels

CONCLUSIONS

The objective of this study was to assess several 'Test' platelet rich plasma (PRP) releasate preparation methods and determine the PDGF concentration of each releasate sample.

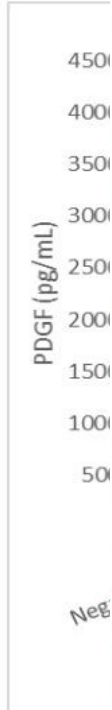
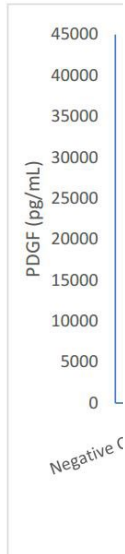
The mean platelet recovery was 56% and the mean platelet concentration factor was 5.1 times baseline. These findings met the acceptance criteria outlined by Sponsor for a 4 – 6x concentration factor above baseline.

The Osmotic Lysis (OL) method and the Autologous Thrombin (AT) method yielded the lowest PDGF concentrations of the Test methods, at 15,297 pg/mL and 16,674 pg/mL, respectively. The Freeze-Thaw (FT) and AT + CaCl₂ methods were comparable and resulted in higher PDGF concentrations at 20,499 pg/mL and 19,692 pg/mL, respectively (Table II, Figures I – II).

The study data demonstrates that the OL and AT PRP releasate preparation methods were the least effective methods for platelet activation and PDGF release. Both FT and AT + CaCl₂ releasate preparation Test methods resulted in substantial PDGF release, which were 53% and 51%, respectively, of that measured for the positive control method.

Figure II. Individual Method

Figure I. I



Lumbar FSU Approach PL @ TF and IL Sites

- Facets
- Muscles
- Ligaments
- ***Nerve roots receive PL
- SI Joint + Ligs
- Bilateral Approach for most!
- Glutes/Hip (sometimes)



Platelet Releasate – Disc (Akeda)

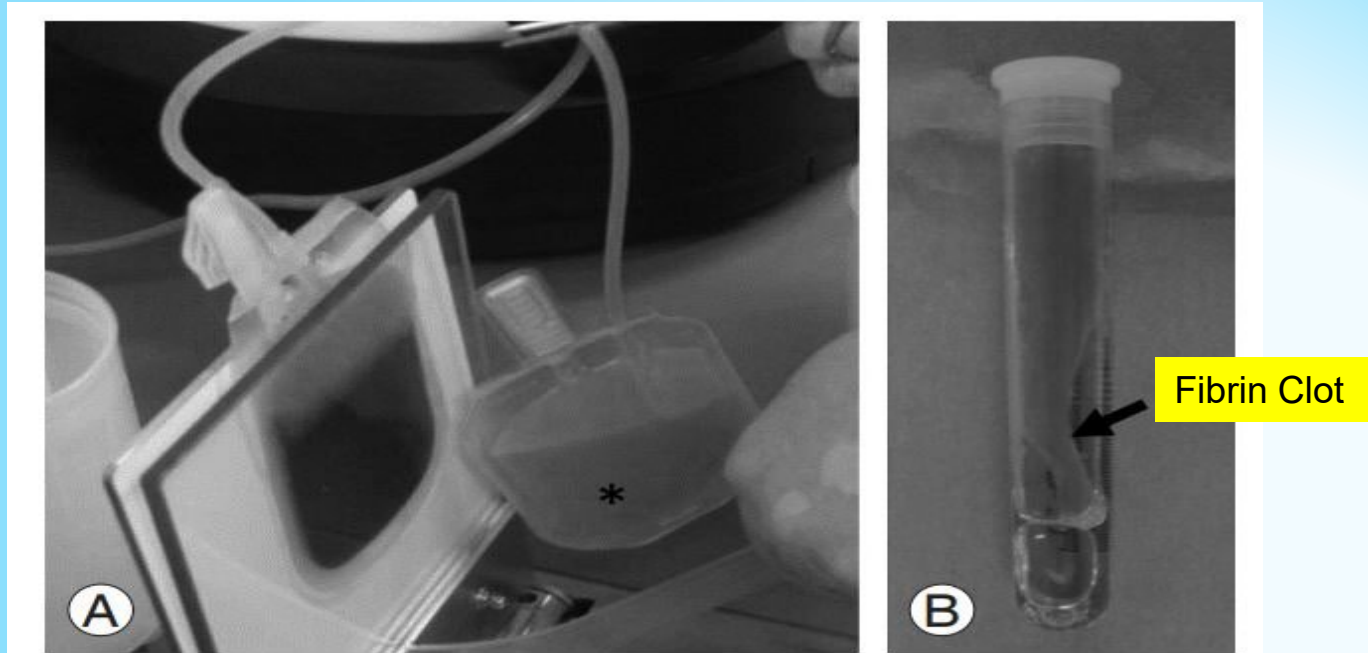
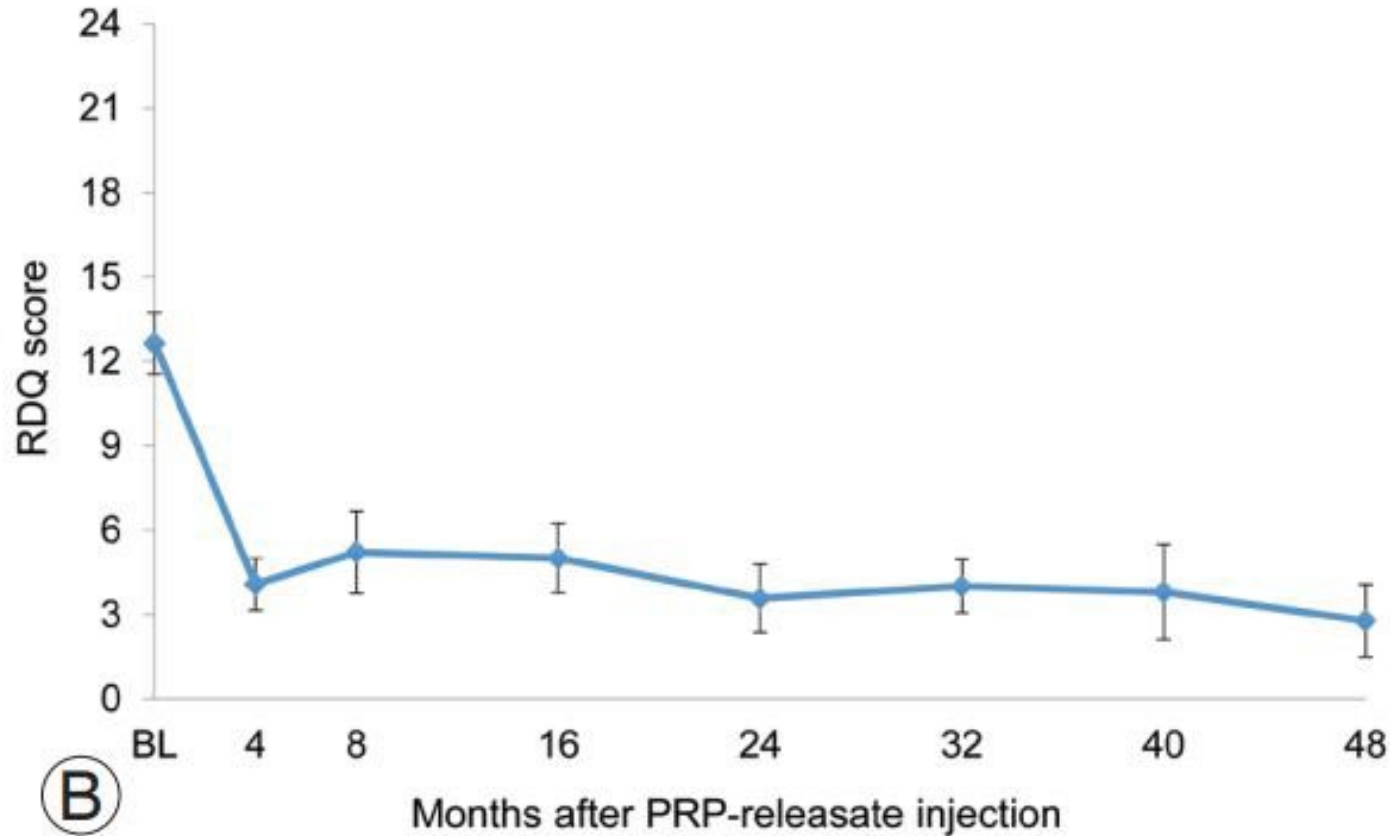
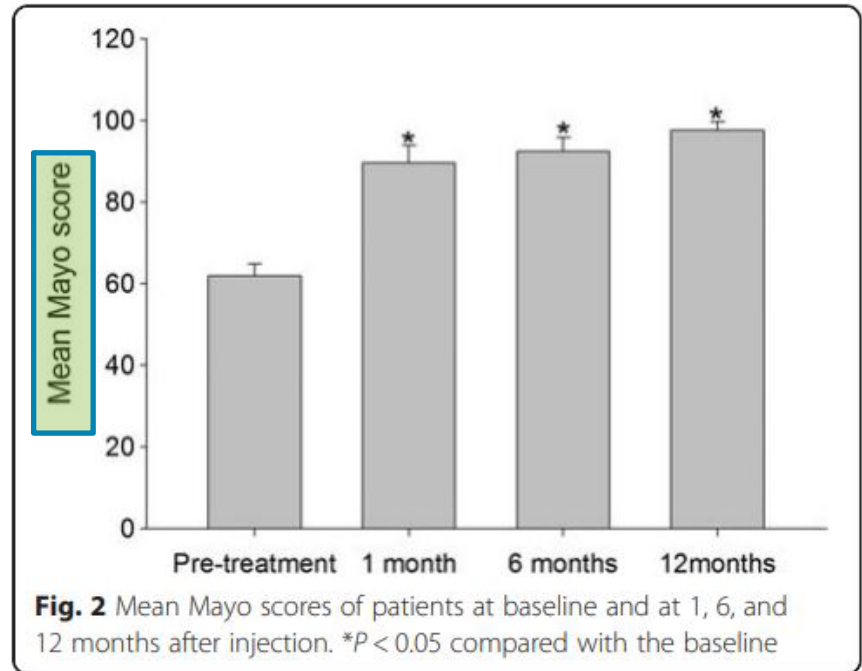
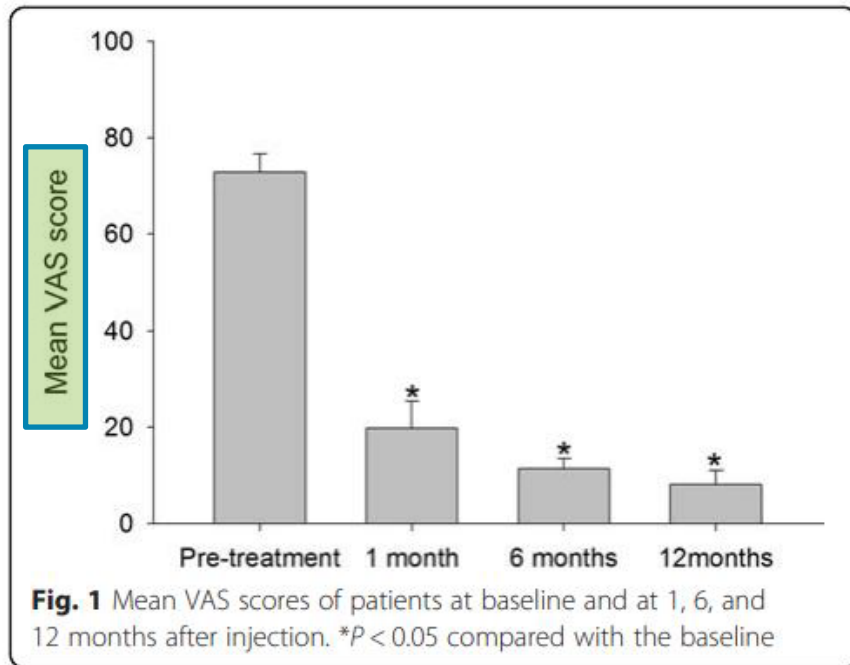


Fig. 1. Preparation of platelet-rich plasma (PRP) releasate. **(A)** Following the two-stage centrifugation of whole blood, PRP (asterisk) was transferred to a storage bag using a sterile blood collection bag system. **(B)** A mixture of autologous serum and 2% CaCl₂ was added to PRP for clot (gel) formation (arrow).

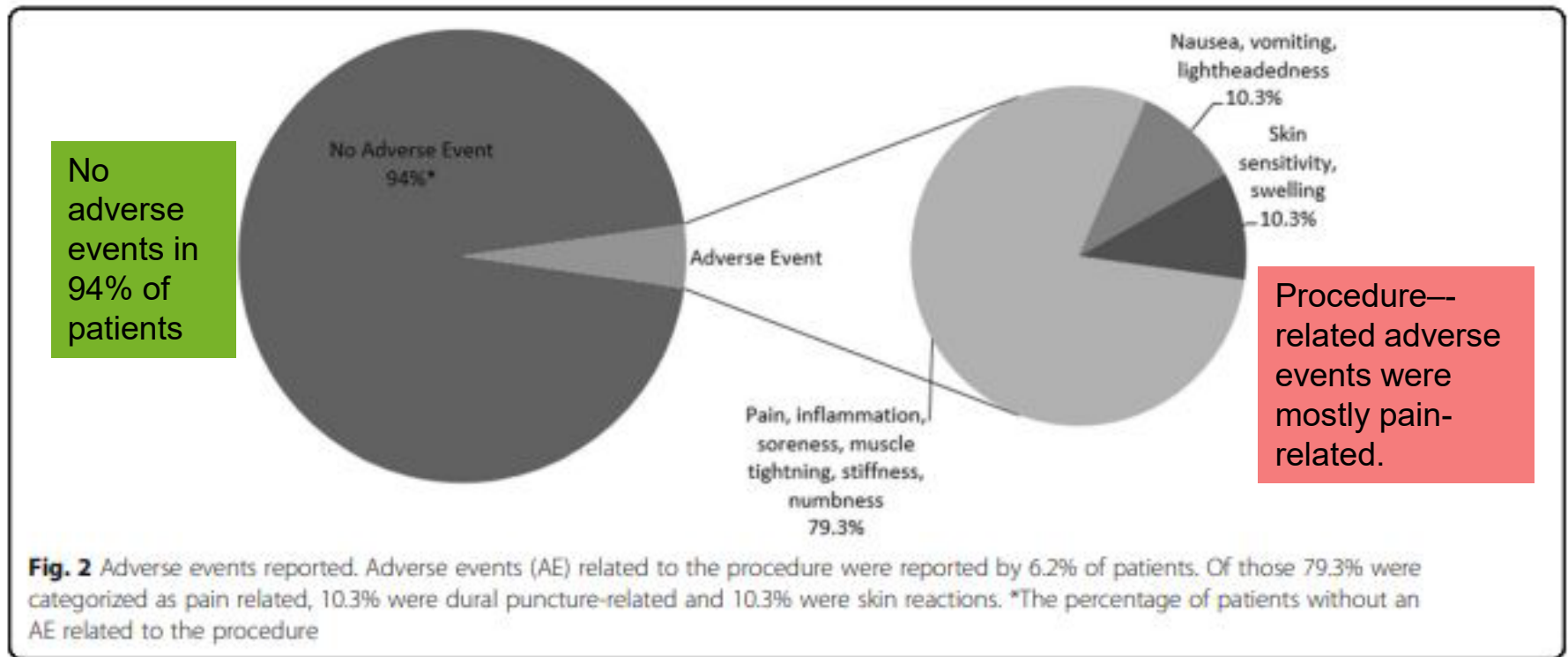
Platelet Releaseate – Disc (Akeda)



Platelet Lysate – Elbow (Tan)



Platelet Lysate – Radiculopathy (Centeno)



Platelet Lysate – Radiculopathy (Centeno)

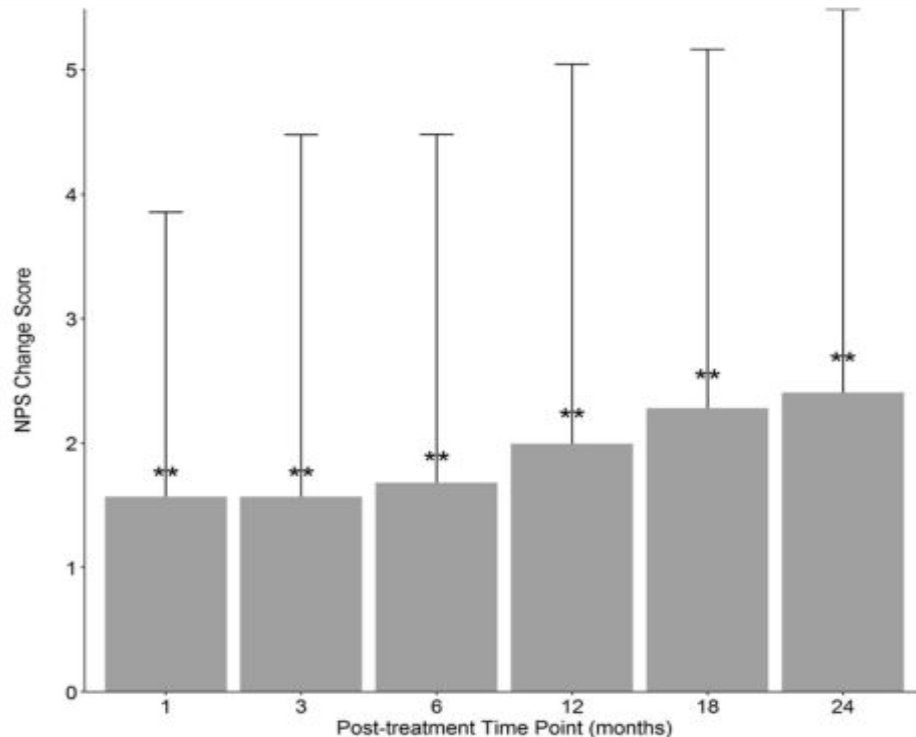


Fig. 3 NPS average change scores. Numeric pain score (NPS) average change from baseline to post-treatment with standard deviation. Number of patients reporting at each time point: 1-month ($N = 139$); 3-month ($N = 192$); 6-month ($N = 181$); 12-month ($N = 174$); 18-month ($N = 143$); 24-month ($N = 126$). Statistical comparisons are to baseline. ** $p < .0001$.

Improvements in
pain scores
compared to
baseline

Centeno, J Exp
Orth 2017

Platelet Lysate – Radiculopathy (Centeno)

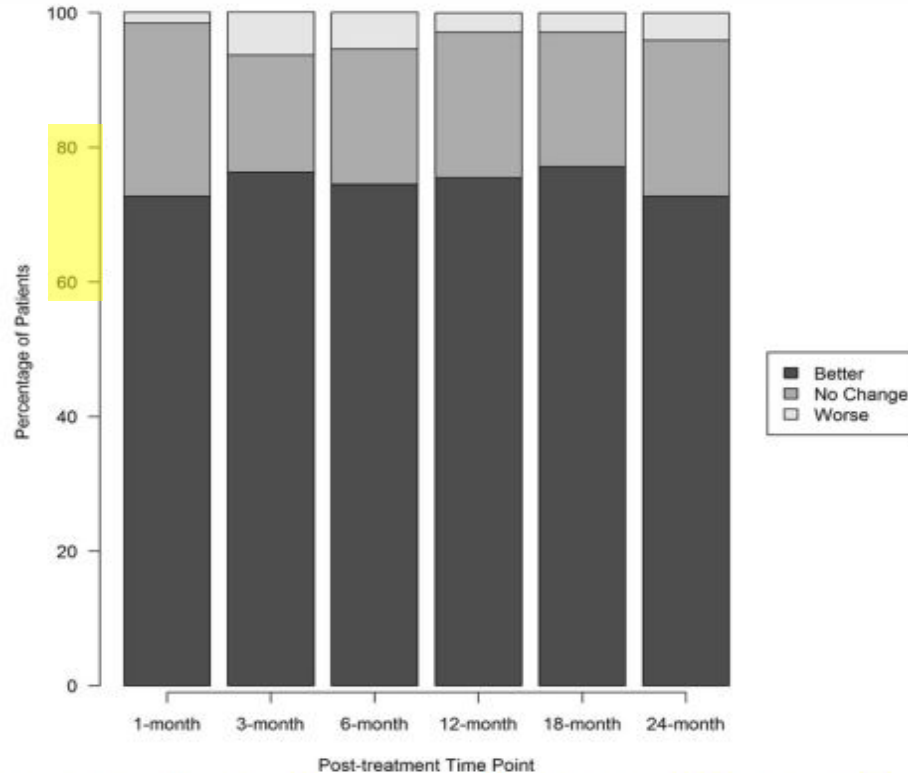


Fig. 4 Modified SANE ratings tier plot. The percent of total patients at each post-treatment time point reporting feeling better (modified SANE > 0), no change (modified SANE = 0), or worse (modified SANE < 0). Patients reporting at each time point: 1- month (N = 128); 3-month (N = 211), 6-month (N = 216), 12-month (N = 203), 18-month (N = 153), 24-month (N = 129)

70+ % of patients reported feeling better at all time points

Centeno, J Exp Orth 2017

Platelet Lysate – Radiculopathy (Centeno)

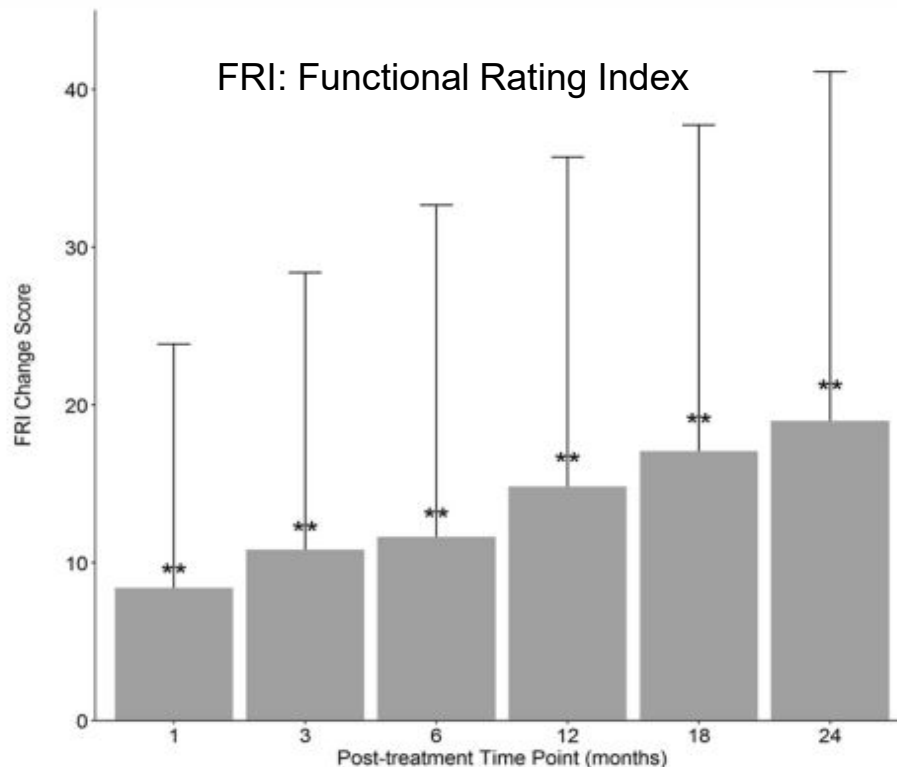


Fig. 5 FRI average change scores. Functional rating index (FRI) averaged change in scores from baseline at each post-treatment time point with standard deviation. The number of patients reporting at each time point: 1-month ($N = 111$); 3-month ($N = 144$), 6-month ($N = 146$), 12-month ($N = 136$), 18-month ($N = 114$), 24-month ($N = 100$). ** $p < .0001$

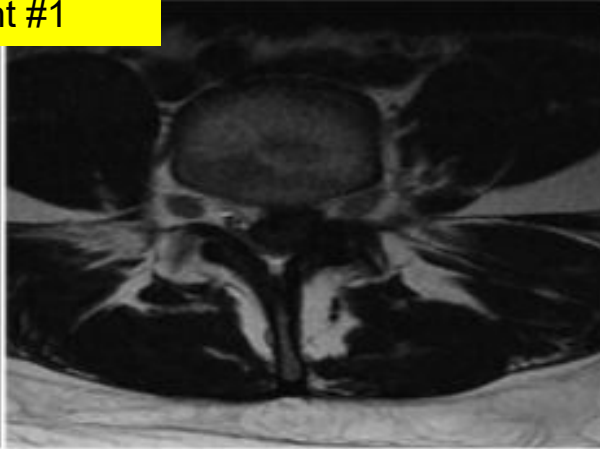
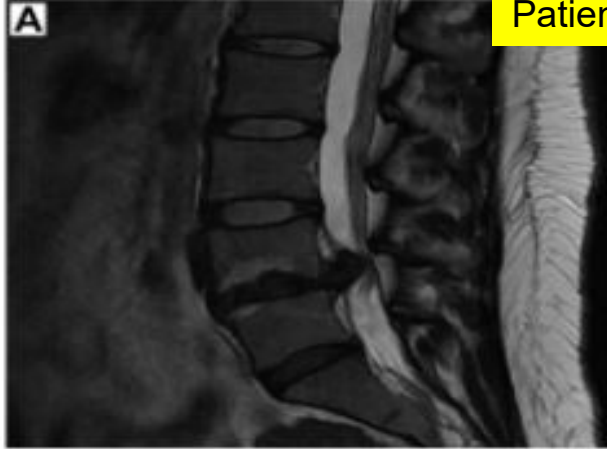
MCID for FRI
(9 Points)
Met or exceeded
at all time points
beyond 1 month

**Centeno, J Exp
Orth 2017**

Platelet Lysate – HNP (Rawson)

A

Patient #1

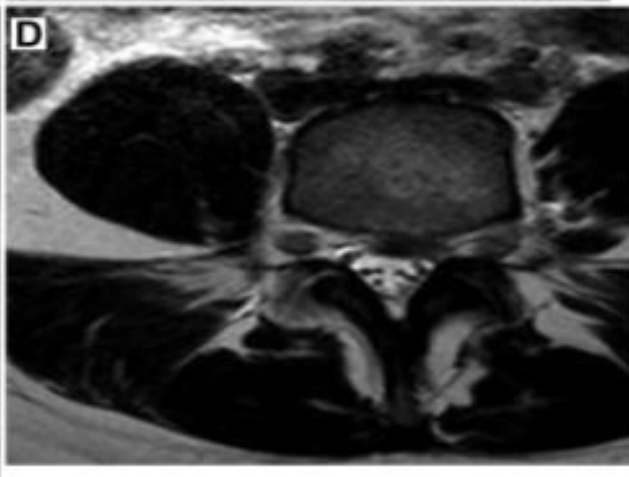


Baseline

C



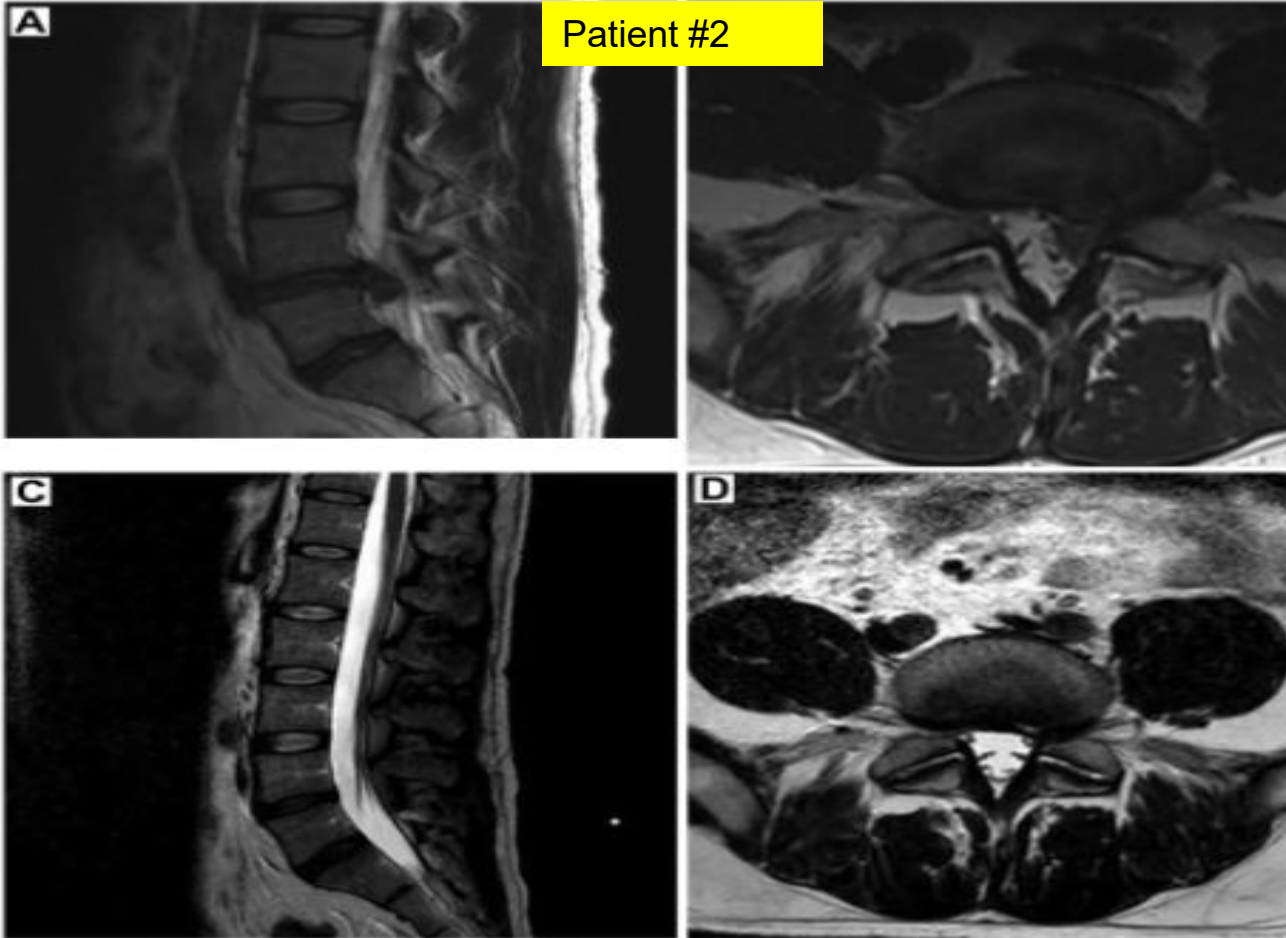
D



8 weeks;
(4 wks s/p
PLE #2)

Rawson, J
Am Osteo
Assoc 2020

Platelet Lysate – HNP (Rawson)



Patient #2

Baseline

20 weeks;
(12 wks s/p
PLE #2)

Rawson, J
Am Osteo
Assoc 2020

ONFH (Surgeon)

Bilat
RCT and
KL 2 OA

These freaks
need
Orthobiologics

Future Split Tear
Peroneus Brevis
(Too Young/Unaware)

Back, SI Joint





Thank You!

[Email: jknab@portcitypain.com](mailto:jknab@portcitypain.com)

LinkedIn: @John Knab MD

Text/Cell: (910) 352-2515

www.portcitypain.com

Selected References

1. Strandberg G, Sellberg F, Sommar P, Ronaghi M, Lubenow N, Knutson F, Berglund D. Standardizing the freeze-thaw preparation of growth factors from platelet lysate. *Transfusion*. 2017 Apr;57(4):1058-1065. doi: 10.1111/trf.13998. Epub 2017 Feb 9. PMID: 28182293.
2. da Fonseca L, Santos GS, Huber SC, Setti TM, Setti T, Lana JF. Human platelet lysate - A potent (and overlooked) orthobiologic. *J Clin Orthop Trauma*. 2021 Jul 28;21:101534. doi: 10.1016/j.jcot.2021.101534. PMID: 34386346; PMCID: PMC8339333.
3. Gruba, Sarah M. Danielle H. Francis, Audrey F. Meyer, Eleni Spanolios, Jiayi He, Ben M. Meyer, Donghyuk Kim, Kang Xiong-Hang, and Christy L. Haynes Characterization of the Presence and Function of Platelet Opioid Receptors *ACS Measurement Science Au* 2022 2 (1), 4-13 DOI: 10.1021/acsmeasuresciau.1c00012
4. Bernardi M, Albiero E, Alghisi A, Chierigato K, Lievore C, Madeo D, et al. Production of human platelet lysate by use of ultrasound for ex vivo expansion of human bone marrow-derived mesenchymal stromal cells. *Cytotherapy*. 2013;15(8):920–9. doi:10.1016/j.jcyt.2013.01.219
5. Rawson B. Platelet-Rich Plasma and Epidural Platelet Lysate: Novel Treatment for Lumbar Disk Herniation. *J Am Osteopath Assoc*. 2020 Mar 1;120(3):201-207. doi: 10.7556/jaoa.2020.032. PMID: 32091562.
6. Hara, Y., M. Steiner, and M. G. Baldini. "Platelets as a source of growth-promoting factor (s) for tumor cells." ***Cancer Research* 40.4 (1980): 1212-1216.**
7. Doucet C, Ernou I, Zhang Y, et al. Platelet lysates promote mesenchymal stem cell expansion: a safety substitute for animal serum in cell-based therapy applications. *J Cell Physiol*. 2005. <https://doi.org/10.1002/jcp.20391>.
8. Astori G, Amati E, Bambi F, et al. Platelet lysate as a substitute for animal serum for the ex-vivo expansion of mesenchymal stem/stromal cells: present and future. *Stem Cell Res Ther*. 2016.
9. Centeno C, Markle J, Dodson E, et al. The use of lumbar epidural injection of platelet lysate for treatment of radicular pain. *J Exp Orthop*. 2017. <https://doi.org/10.1186/s40634-017-0113-5>.
10. Akeda K, Ohishi K, Masuda K, Bae WC, Takegami N, Yamada J, Nakamura T, Sakakibara T, Kasai Y, Sudo A. Intradiscal Injection of Autologous Platelet-Rich Plasma Releasate to Treat Discogenic Low Back Pain: A Preliminary Clinical Trial. *Asian Spine J*. 2017 Jun;11(3):380-389. doi: 10.4184/asj.2017.11.3.380. Epub 2017 Jun 15. PMID: 28670405; PMCID: PMC5481592.
11. Andia I, Perez-Valle A, Del Amo C, Maffulli N. Freeze-Drying of Platelet-Rich Plasma: The Quest for Standardization. *Int J Mol Sci*. 2020 Sep 20;21(18):6904. doi: 10.3390/ijms21186904. PMID: 32962283; PMCID: PMC7555364.
12. Tan et al. *Journal of Orthopaedic Surgery and Research* (2016) 11:17 DOI 10.1186/s13018-016-0349-2