CYTKINE EXPRESSION IN DEGENERATIVE ROTATOR CUFF TEARS:

IT IS NOT ALL ABOUT MECHANICS, THINK ABOUT BIOLOGY!

Michell Ruiz-Suárez MD, PhD

Traumatología Deportiva de México, S.C.
Instituto Nacional de Rehabilitación
Mexico City, Mexico
Disclosure

• Consultant – ConMed Linvatec

• Chair, Scientific and Program Committee – Asociación Mexicana de Cirugía Reconstructiva Articular y Artroscopia (AMECRA)

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What happens after a rotator cuff repair?

- Three possible outcomes:
  1. Tendon healing
  2. Failure to heal
  3. Retear
Let’s focus on…

1. Failure to heal

2. Retear

Are they different entities?

How can we determine a retear was not actually a failure to heal?
Can we evaluate rotator cuff healing?

• At the present time we can only assume a tendon has healed if the musculotendinous unit remains on top of the greater tuberosity in imaging studies (ultrasound/MRI) through time

• Nevertheless, we do not have a reliable method that can determine there is a transition tissue (fibrovascular scar/enthesis) between the rotator cuff tendons and the greater tuberosity
What we currently know…

If rotator cuff remains on top of the greater tuberosity (healing?), it correlates with better clinical outcomes than those that are not.
As surgeons, how can we increase our success rate?

• First, let’s define what is success with regard to RCR

  1. Clinical outcomes? (pain, range of motion, return to activities, strength)

  2. Tendon healing?
Better clinical outcomes

• Short to mid-term good results

• Clinical outcome may not be constant through time

• Patients may not be as happy in the long-term as they were in the short to mid-term recovery period

• Strength is almost never recovered at normal values (not even at age/gender normalized values)
Therefore...

- Probably, not all tendons that sit on top of the greater tuberosity have actually healed, they are just sitting there forced by our repair construct.

- We are aiming to increase our healing rates.
And we have our strategies

• But, almost all our strategies have been direct to increase stiffness of our construct, mechanical load to failure, contact area, and pressure area

• How?
  
  • Modification of suture anchor materials and suture anchor designs
  
  • Use of super strength suture materials
  
  • Modifying the diameter and structure of these suture materials
  
  • Using repair configurations that increase contact/pressure areas on the greater tuberosity
Have these strategies worked?
Have these strategies worked?

Partially
Have these strategies worked

- Clinical outcomes are very similar between single and double or more row-configurations

- Tying knots medially has not demonstrated significant differences

- Use of super strength suture materials can actually harm the tendon and limit their vascular flow

- Increasing contact area and/or pressure has not increased success rates (in terms of clinical results)
• We have been very good at designing mechanical improvements

• We have even demonstrated that at time-zero, and in cadavers they work great!

• “Unfortunately”, the tendon is a living tissue, and biology does not care too much about time zero results
Since mechanical improvements have not reported significant improvements, now what?

Modify the biological response to healing:

- Platelet rich plasma
- Platelet rich matrix
- Mesenchymal stem cells
- Biological/synthetic augmentation patches
- Botulinum toxin A
- Modification of metalloproteinases levels through chelants (doxycycline)
- Etc, etc, etc
Have these strategies worked?
Have these strategies worked?

Partially
Due to intrinsic differences in shoulder anatomy and physiology among species, translation to human shoulder pathology is difficult.
In other words..

• Our improvements in biomechanics have not been enough

• Our biological modifications to a healing response have not been enough

• The application of translational science from animal models has not proven to increase our understanding of the pathology
One important principle has been overlooked…

Know your enemy

Know your enemy and know yourself and you can fight a hundred battles without disaster.

(Sun Tzu)
What is happens to tendon histology after a degenerative tear?

- Fragmentation and disorientation of collagen fibrils (Matthews TH, JBJS, 2006)

- Decrease tenocyte number and phenotype modification (Maffulli N, Disabil Rehabil, 2008)

- No evidence there is inflammatory cell migration (Hashimoto T, CORR, 2003)

- Limited vascular supply (Millar NL, AJSM, 2010)
What is happens to tendon histology after a degenerative tear?

• These are the tendon findings, but let’s not forget it is only one element of the musculotendinous unit
Fatty infiltration and muscle atrophy

- Once there is a complete tendon injury, there is muscle denervation.

- Fatty infiltration may originate through hypertrophy of preexistent adipocytes, mesenchymal stem cell differentiation on- or off-site.

- PPAR (peroxisome proliferated-activated receptors) and C/EBP (CCAAT enhancer-binding proteins) via may condition a transformation from miocytes to adipocytes.

- Fatty infiltration correlates with age, symptoms duration and tear size.
Fatty infiltration and muscle atrophy

- The degree of fatty infiltration correlates with failure-to-heal rate

- Degree of fatty infiltration inversely correlates with clinical improvement after surgery
• So now we know a little bit about repair mechanics, tear biology

• Still, we have very little information about tendon physiology
Our attempt to clarify the physiology of degenerative tears

- Cross-sectional comparison of two groups based on fatty infiltration at the time of surgery (MRI- Zanettii´s tangent sign)
  - Group 1: < 50% of fatty infiltration
  - Group 2: > 50% of fatty infiltration

- Inclusion criteria: Degenerative rotator cuff tears, >40 years, both genders

- Exclusion criteria: Comorbidities that may modify inflammatory response, prior surgery, steroid/NSAID infiltration
Cytokine assessment

- Multiplex (MagPix, BioRad)
- Hybrid system ELISA and flow cytometry
- Th1 and Th2 response
- Cytokines responsive to inflammation

- IL-1α
- IL-1β
- TNF-α
- IL-2
- IL-4
- IL-6
- IL-8
- IL-1RA
- sIL-2RA
- IL-10
- IL-12(p70)
- IL-17
- Eotaxin
- IFN-γ
- IP-10
- MCP-1
- MIP-1α
- MIP-1β
- VEGF
- Leptin
- MMP-9
Results

We included 25 patients:

Group 1: 13
Group 2: 12

Group 1: 61.7 ± 7.1 years
Group 2: 66 ± 10.5 years

**Constant-Murley**
Group 1: 48.2 ± 20.1 pts
Group 2: 38.3 ± 17.3 pts
p=0.44

**Simple Shoulder Test**
Group 1: 3.7 ± 1.2 pts
Group 2: 1.6 ± 1.5 pts
p=0.03

**DASH**
Group 1: 66.4 ± 19.5 pts
Group 2: 65.5 ± 16.7 pts
p=0.93
Results

• Cytokine profile (IL-4)

\[ p = 0.005 \]
Results

• Cytokine profile expression (IL-10)

IL-10

Group 1

Group 2

p=0.022
Results

- Cytokine profile expression (IL-12p70)

\[
\text{IL-12p70}
\]

\[p=0.022\]
Results

- Cytokine profile expression (all patients)
Results

Cytokine profile expression (MIP-1β)

Group 1 vs Group 2

MIP-1b

p=0.005
Results

Cytokine profile expression (VEGF)

Group 1

Group 2

p=0.008
Results

- We found no other significant differences in IFN-γ, IL-2, or IL-6

- Does not mean they are not present

- In other words, they do not seem to play a significant role in the biology of the disease
Results

Correlation between cytokines (Pearson coefficient)

**Group 1**
- IL-4 e IL-12p-70, r=0.911
- IFN\(\gamma\) e IL-10, r=-0.11
- IL-4 e IL-10, r=-0.19
- IL-10 e IL-12p70, r=-0.47

**Group 2**
- IFN\(\gamma\) e IL-4, r=0.99
- IFN\(\gamma\) e IL-10, r=0.95
- IL-4 e IL-10, r=0.95
Biochemical implications of our results

- Fatty infiltration does correlate with cytokine expression (due to the study design we cannot say there is a cause-effect relationship)
- No patient had an acute inflammatory response at the time of surgery
Biochemical implications of our results

• In patients with <50% of fatty infiltration there was only a significant presence of a pro-inflammatory cytokine: IL-12p70

• This group also showed presence of IL-4, it appears that the inflammatory response may be shifting to an anti-inflammatory response

• Since we studied Th1 and Th2 systems simultaneously and not as independent cytokines, antiinflammatory response seems to be predominant in degenerative rotator cuff tears
Biochemical implications of our results

• IL-10 is the cytokine with the highest concentration, particularly in patients with >50% fatty infiltration

• In this group there is a significant present of VEGF (hypertrophic fibrovascular tissue)

• Significant concentration of MIP-1β (may explain more pain in this group)
Biochemical implications of our results

- IL-4 inhibits the effect of IL-12, once this is established, the concentration of IL-10 rapidly increases.

- Recent studies have suggested that a Th2 response may increase the levels of VEGF.

- MIP-1β (CCL-4) has been correlated with pain in chronic soft tissue injury.
Conclusions

- Is difficult for a diseased tissue to transform to a healthy one, just because my surgical technique was perfect and added growth factors.

- If you want to modulate the healing response, you HAVE to know (micro) environment of the damaged tissue.

- We have to understand that biology works as a system.

- We cannot study interleukins or growth factor as isolated factors.
Future directions

• Investigate if surgery can reverse the antiinflammatory effect of IL-10

• Effect of MSC in antiinflammatory environment

• Effect of addition of pro-inflammatory factor in the environment of prevalent IL-10
Conclusions

• It is not only about mechanics

• It is not only about biology

• It is about understanding we are dealing with biological systems that are immerse in complexity
Thank you