Urinary CTX-II, Serum MMP-3 And Serum PIIANP Following Anterior Cruciate Ligament Reconstruction: A Pilot Study

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Disclosures

- Lachlan Batty
  - Speaker for Arthrex, Device Technologies, Smith and Nephew
  - Paid Consultant for Arthrex
  - Support received from Smith and Nephew
- Kate Webster
  - Nil
- Brian Devitt
  - Speaker for Arthrex
  - Support received from Smith and Nephew
- Jereme Spiers
  - Nil
- Haydn Klemm
  - Nil
- Timothy Whitehead
  - Speaker for Smith and Nephew, Arthrex, Medacta
  - Paid Consultant for Medacta
  - Support received from Smith and Nephew
- Andrew Hill
  - Nil
- Julian Feller
  - Nil

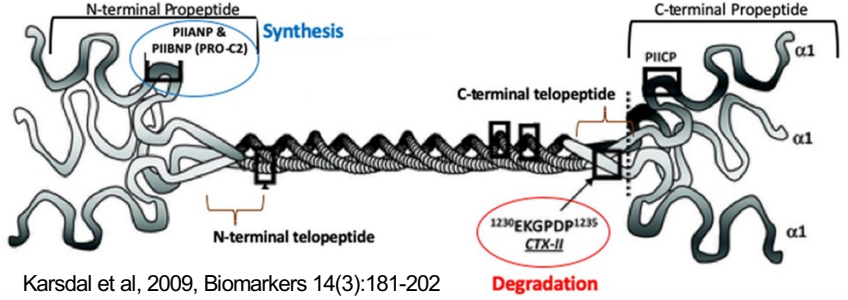
Background

- Post-traumatic osteoarthritis (OA) is a well-recognized occurrence following anterior cruciate ligament (ACL) injury and ACL reconstruction (ACLR).[1]
- Synovial fluid sampling studies suggest changes occur at the cellular level soon after ACL injury and surgery.[2]
- Local (synovial) and systemic (serum/urine) biomarkers may have prognostic value in identifying patients at risk of post traumatic OA before clinical or radiological signs.
- Urinary and serological biomarkers are advantageous clinically as they avoid the need for arthrocentesis.

Background – Biomarkers of interest

- Urinary C-terminal cross-linked telopeptide of type II collagen (**CTX-II**)
  - By-product of articular cartilage degradation and a potential measure of type 2 collagen breakdown
- Serum N-propeptide of collagen IIA (**PIIANP**)
  - A splice from type II collagen synthesis and a potential measure of type 2 collagen production
- Serum Matrix Metalloproteinase 3 (**MMP-3**)
  - Extracellular enzyme involved in chondral metabolism and degradation of extra cellular matrix components

Type 2 Collagen[3]



Karsdal et al, 2009, Biomarkers 14(3):181-202

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Research Questions

- Can urinary CTX-II, serum PIIANP and serum MMP3 be measured post ACL reconstruction?
- Do levels of these three biomarkers change during the first-year after ACL reconstruction?
- Is there an association between biomarker levels and patient age, BMI or time from injury to surgery?

Methods

- Pilot of 22 patients from a prospective longitudinal study of 683 patients undergoing ACLR
- Stored serum and urine samples kept in -80°C freezer
  - Samples taken at baseline (immediately prior to surgery), 6- and 12-months post operatively
- Commercially available, pre-clinical ELISA assays
  - **Urinary CTX-II:** CloudClone CTX-II ELISA; Cloud Clone Corporation, TX, USA, and CartiLaps® IDAC10F1 ELISA; Immunodiagnostic Systems (IDS) Holdings Ltd, United Kingdom, (both normalized to urinary Creatinine)
  - **Serum MMP3:** ELH-MMP3; RayBiotech Life Inc, Peachtree Corners, GA
  - **Serum PIIANP:** MBS109368; MyBioSource, San Diego, CA
- Statistical analysis
  - Pearson correlation coefficients and one way ANOVA

Results – Patient demographics

		n = 22
Age (years)		25.24 (7.96)
Male sex, n (%)		12 (54.54%)
Right, n (%)		15 (68.18%)
Time to surgery (days)		294.59 (411.11)
		Median 152 (IQR 59.0-278.5)
		Range 10 - 1513
Graft, n (%)	Hamstring autograft	20 (90.91%)
	Quadricep autograft	1 (4.55%)
	Patella tendon autograft	1 (4.55%)
Body Mass Index (BMI)		25.13 (3.66)

Data presented as Mean (SD) unless otherwise stated

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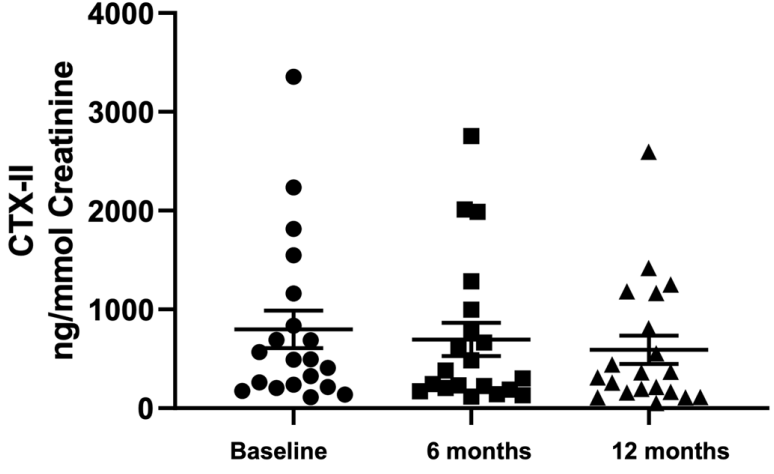
Results – CTX-II (CartiLaps Assay)

- No significant change with time
  - (F(2, 40) = 1.599, p=0.215, eta2 = 0.074)
- Negative correlation to patient age
  - Baseline (r= -0.844, p<0.001), 6 months (r=-0.830, p<0.001), 12 months (r= -0.747, p<0.001)
- No correlation to BMI
  - Baseline (r=-0.316, p=0.163), 6 months (r=-0.243, p=0.289), 12 months (r=-0.165, p=0.475)
- No correlation to time to surgery
  - Baseline (r= -0.283, p=0.214), 6 months (r= -0.238, p=0.300), 12 months (r= -0.206, p= 0.369)



- 1 extreme outlier excluded
- Error bars represent mean and standard error

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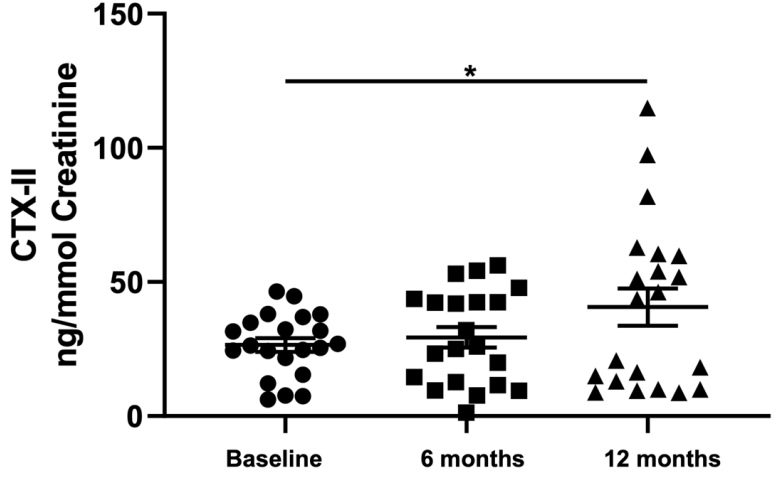
Results – CTX-II (CloudClone Assay)

- CTXII levels increased with time
  - ($F_{(2,40)}$ = 3.65, p=0.035, eta2 = 0.154)
- No correlation to age
  - Baseline (r=0.024, p=0.918), 6 months (r=-0.068, p=0.771), 12 months (r=0.103, p=0.656)
- No correlation to BMI
  - Baseline (r=-0.095, p=0.683), 6 months (r=-0.170, p=0.461), 12 months (r=0.432, p=0.050)
- No correlation to time to surgery
  - Baseline (r= 0.190 ,p=0.410), 6 months (r=0.064, p=0.784), 12 months (r=0.277, p=0.225)
- **CTX-II levels differed between the CloudClone and CartiLaps ELISA at all timepoints (p ≤0.004)**



- 1 patient excluded as missing 12-month data
- Error bars represent mean and standard error
- * Significant at p≤ 0.05 level

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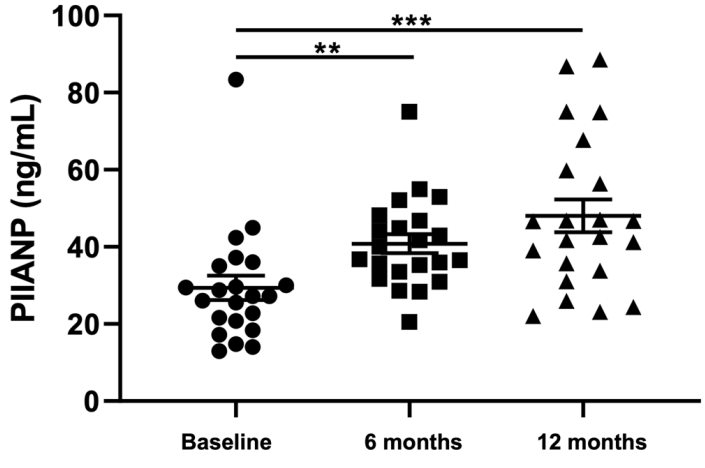
Results – PIIANP

- PIIANP levels increased with time
  - (F(2,40) = 13.03, p<0.001, eta2 = 0.395) (Figure 4)
- No correlation to age
  - Baseline (r=-0163., p=0.480), 6 months (r=0.142, p=0.538), 12 months (r=-0.059, p=0.798)
- No correlation between BMI
  - Baseline (r=-0.102, p=0.661), 6 months (r=0.004, p=0.986), 12 months (r=-0.148, p=0.521)
- No correlation to time to surgery
  - Baseline (r= 0.075,p=0.748), 6 months (r= -0.323, p=0.153), 12 months (r= 0.014, p=0.951)



- 1 extreme outlier excluded
- Error bars represent mean and standard error
- ** significant at p≤ 0.01 level
- *** significant at p≤ 0.001 level

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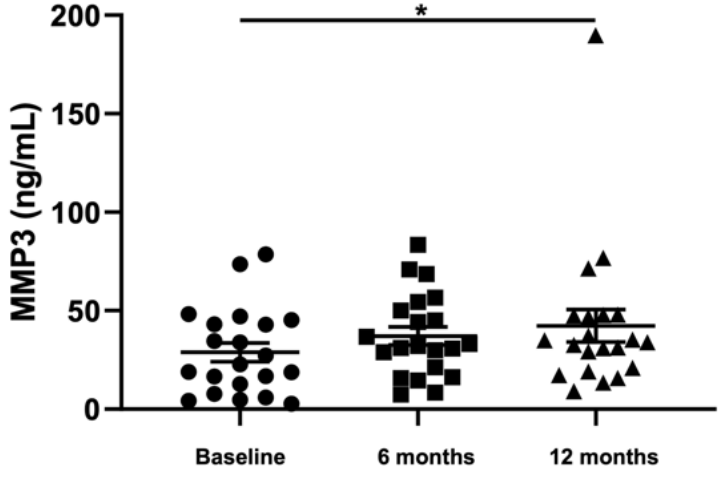
Results – MMP-3

- MMP3 levels increased with time
  - (F(2,38) = 4.029, p=0.026, eta2 = 0.175)
- No correlation to age
  - Baseline (r=0.327, p=0.160), 6 months (r=0.174, p=0.464) 12 months (r= - 0.113, p=0.635)
- No correlation to BMI
  - Baseline (r=0.235, p=0.319), 6 months (r=-0.049, p=0.838), 12 months (r=-0.082, p=0.731)
- No correlation to time to surgery
  - Baseline (r=-0.180,p=0.447), 6 months (r=-0.103, p=0.665), 12 months (r=-0.065, p=0.784)



- Two patients identified as extreme outliers excluded
- Error bars represent mean and standard error
- * significant at the p≤0.05 level

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Conclusions

- Systemic markers of chondral metabolism can be measured after ACL reconstruction
- Systemic biomarker levels change during the first 12 months post surgery
  - A decrease in CTX-II levels (CartiLaps Assay) potentially indicates reduced type 2 collagen degradation
  - An increase in PIIANP levels potentially indicates increased synthesis of type 2 collagen
  - Together, these data suggest ongoing alterations in chondral homeostasis, with a possible anabolic effect
- The specific assay used is critical to consider when interpreting CTX-II levels and comparing data

References

1. Cinque ME, Dornan GJ, Chahla J, Moatshe G, LaPrade RF. High rates of osteoarthritis develop after anterior cruciate ligament surgery: an analysis of 4108 patients. The American journal of sports medicine 2018; 46: 2011-2019.
2. Kaplan DJ, Cuellar VG, Jazrawi LM, Strauss EJ. Biomarker Changes in Anterior Cruciate Ligament-Deficient Knees Compared With Healthy Controls. Arthroscopy 2017; 33: 1053-1061.
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