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LONGITUDINAL STUDY OF CARTILAGE INTERMEDIATE LAYER PROTEIN  
AND CARTILAGE OLIGOMERIC MATRIX PROTEIN OVER 5 YEARS AFTER ANTERIOR CRUCIATE  
LIGAMENT RUPTURE

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*Abstract:*

**Purpose:** Knee trauma induces joint cartilage degradation and plays an important role in the development of osteoarthritis (OA). Our study aimed to explore the serum levels of the cartilage biomarkers Cartilage Intermediate Layer Protein (CILP) and Cartilage Oligomeric Matrix Protein (COMP) over 5 years in the longitudinal randomized control study (KANON), initiated to investigate structured rehabilitation of anterior crucial ligament (ACL) injuries with or without surgical reconstruction.

**Methods:** Blood samples were obtained at baseline (0-6 weeks after injury), 3, 6, 12, 24 and 60 months after injury) from 121 subjects (26% women, mean age of 26 years, range 18-36 years) with an acute ACL rupture to a previously un-injured knee. Reference samples were obtained from 21 age-matched knee-healthy individuals. Serum concentrations of the biomarkers were measured with a commercially available sandwich COMP ELISA (AnaMar AB) and an in-house research competitive immunoassay for CILP (AnaMar AB), where a 60 amino acid long synthetic peptide (C-terminal part of CILP domain 1) was used as coat and a peroxidase-conjugated polyclonal goat anti-CILP was used for detection. Mann-Whitney rank sum test was used to compare COMP and CILP concentrations at different time points between injured and knee-healthy subjects, and Spearman's rank order correlation ( $r_s$ ) for assessment of correlation.

**Results:** Despite variation in the concentration of serum CILP in individual ACL patients over time, the median serum concentrations at the cohort level were stable over the 5-year period, although the CILP level was significantly increased compared to knee-healthy subjects at all time points (Figure 1a). The median serum concentration of COMP in ACL patients showed no significant difference compared to the reference group, and the median concentration showed only small variations over time. However, at each time point after baseline a number of patients showed higher serum COMP levels than any of the knee-healthy subjects though no significant difference was observed when comparing the entire populations (Figure 1b). There was no correlation between CILP and COMP values at the different time points after injury, although a negative correlation ( $r_s = -0.202$ ,  $p = 0.0264$ ) was found for the entire 5-year time period.

**Conclusions:** This is the first time that data are presented for the two serum biomarkers CILP and COMP in a longitudinal cohort of patients having an acute ACL rupture. Serum CILP stays elevated compared to the knee-healthy subjects over the first 5 years after ACL injury, whereas no significant differences could be seen at the population level in serum COMP. Further evaluation, possibly linking biomarker data to MR imaging findings, may shed light on the cartilage matrix remodeling pathways after knee trauma leading to cartilage destruction and OA.

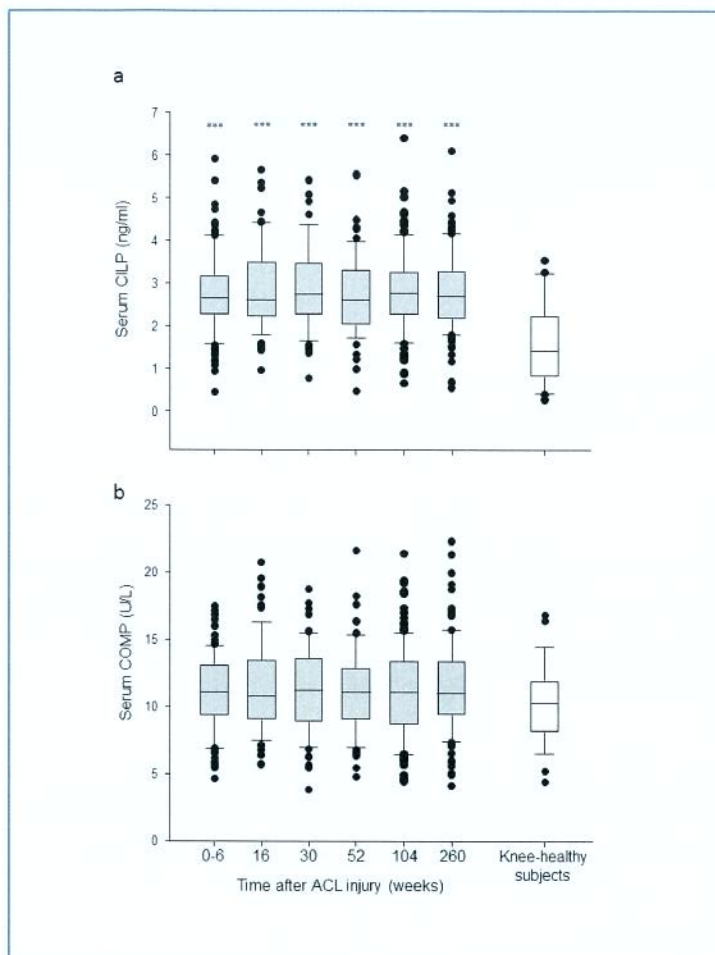


Figure 1: Serum concentrations of CILP (a) and COMP (b) over a 5 year period in ACL injury groups (grey) and in reference group (white). Boxes define group median with 25<sup>th</sup>-75<sup>th</sup> percentile and whiskers 10<sup>th</sup>-90<sup>th</sup> percentile error bars. Circles represent individual outliers. Significance (\*\*\*,  $p < 0.001$ ) compared to references.

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