Limb Idleness Index (LII): A Novel Measurement of Gait Adaptation to Pain in a Rat Model of Osteoarthritis

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INTRODUCTION:
Osteoarthritis (OA) is a debilitating progressive disease manifested as acute pain in joints. The etiopathogenesis of OA is not completely understood. Animal models of OA are established to replicate the pathological changes observed in clinical cases, thus pain measurement is one of the key outcomes. Previous methods utilize paw elevation time (Tonussi CR 1992) or weight-bearing (Bove SE 2003) to measure OA pain. It is assumed that a rat will idle its painful limb by a decrease in the loading time, a decrease on the loading, or a shift of loading to other supporting limbs. As these gait adaptations represent different avoidance mechanisms of mechanical allodynia, it is necessary to combine all these information for a better evaluation of OA pain.

In a rat model of OA by anterior cruciate ligament transaction (ACLT), we measured limb idleness during voluntary ambulation by CatWalk animal gait analysis system. A Limb Idleness Index (LII) is calculated as a product of the ratios of swing duration, target (injured) paw intensity and anchor (supporting) paw intensity. The use of LII to measure OA pain was checked by pharmacological reversal test with buprenorphine injection, and the presence of structural changes related to OA was examined by radiographic and histological analysis.

METHODS:
Animal surgery: The study was approved by the Animal Experimentation Ethics Committee of the authors’ institution. Female SD rats of 12 weeks old (n=12) were randomly divided into ACLT and sham groups. In ACLT group, after medial para-patellar incision and patella dislocation, ACL at right knee was transacted using micro-scissors. Successful transaction was assured by Lachman test. The contralateral left knee remained intact. In sham group, sham operation was performed without transacting the ACL.

Catwalk animal gait analysis: The rats were put for Catwalk analysis at pre-operation, 1 month, 2 months and 3 months post operation. The rats are allowed to walk freely without constraint on speed, internal control for run-to-run variations in walking speed is achieved by normalization to contralateral side. For each trial, 1 to 3 compliant runs with steady ambulation speed (<30% variation) were obtained for further analysis. A Limb Idleness Index (LII) is calculated as a product of the ratios of swing duration (RSW), target paw intensity (RTP) and anchor paw intensity (RAP) (Figure 1). The association of gait changes with pain was assessed by the use of buprenorphine. Catwalk analysis was performed 30 min and 1 day after subcutaneous administration of buprenorphine at 0.025mg/kg body weight. Repeated measure ANOVA was used to detect differences at p<0.05.

RESULTS:

Catwalk gait analysis: At 1, 2, and 3 months, RSW, RTP, and RAP all increased. As a result, LII significantly increased in ACLT group (p<0.01), with 2 months being the highest (Figure 2A). LII decreased 30 min after injection of buprenorphine, and increased again at 1 day after injection (Figure 2B).

Figure 1

![Figure 1](https://example.com/figure1.png)

**Figure 1** Ratio of swing duration (RSW) = Swing duration of RH / Swing duration of LH

**Figure 2**

![Figure 2](https://example.com/figure2.png)

Radiogetic and structural analysis: In ACLT group, there was subchondral bone thinning and decreased bone to tissue volume ratio in tibia (Figure 3). Tibial articular cartilage showed superficial fibrillation and chondrocyte clustering (Figure 3, arrowhead and arrows).

DISCUSSIONS:
This study introduced a new parameter to detect pain-related gait changes in a post-traumatic OA rat model. After ACLT, the rats increased paw elevation time, decreased paw pressure on target limb, and increased paw pressure on the anchor limb. All these resulted in decreased loading of the injured limb. The Limb Idleness Index (LII) was increased after ACLT, and it could be reversed by administration of analgesics, indicating the gait change was pain-related. The presence of subchondral bone thinning, decrease in bone volume ratio, and structural changes indicated characteristic structural changes of OA, which coincided with the gait changes.

Previous study has utilized Catwalk gait analysis system to measure pain in a mono-iodoacetate-induced OA rat model (Ferreira-Gomes J 2008), and only ratio of static paw intensity was found to reflect OA pain. Our study detected pain-related gait change in a post-traumatic OA rat model. After ACLT, the rats increased paw elevation time, decreased paw pressure on target limb, and increased paw pressure on the anchor limb. All these resulted in decreased loading of the injured limb. The Limb Idleness Index (LII) was increased after ACLT, and it could be reversed by administration of analgesics, indicating the gait change was pain-related. The presence of subchondral bone thinning, decrease in bone volume ratio, and structural changes indicated characteristic structural changes of OA, which coincided with the gait changes.

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