

Assessing pain in models of rheumatoid arthritis

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Aim

The aim was to determine how well pain correlated to footpad width using the collagen antibody arthritis model.

Introduction

A hallmark symptom of rheumatoid arthritis in humans is painful swollen joints. Pain can manifest before any inflammation is noticeable^{1,2} as well as persist long after inflammation has resolved.³

In rodent models of arthritis, ankle or footpad width is a commonly used surrogate marker of pain (see Figure 1).

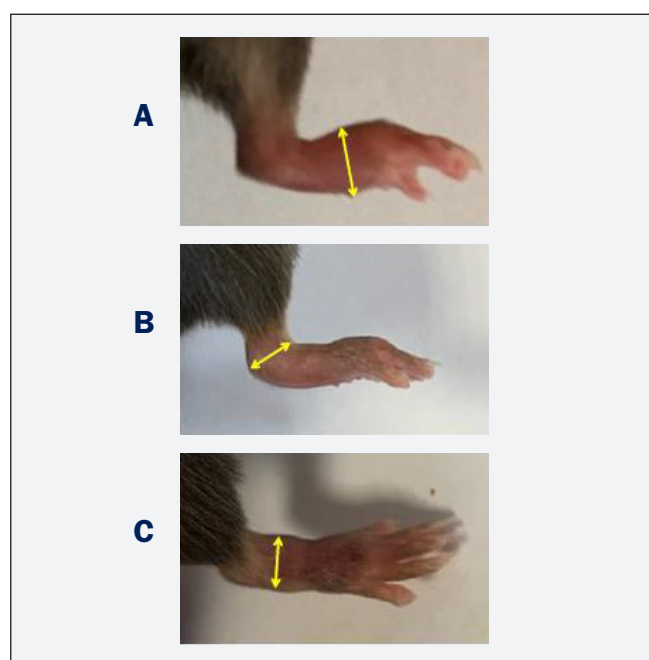


Figure 1. Footpad width as a surrogate measure of pain in arthritis models.

Commonly used methods to assess pain are footpad width (A), ankle width (B) or footpad ankle length (C) (Figure 1).

Techniques to assess pain

Equipment used to measure mechanical withdrawal thresholds (Figure 2) on the left-hand side is an electronic von Frey machine which measures mechanical pain and a circulating water bath set to 2°C that is maintained using ice on the right-hand side.



Figure 2. Equipment

Collagen antibody induced arthritis (CAIA)

A monoclonal antibody (mAb) is injected intravenously (IV) followed by an intraperitoneal (IP) injection of lipopolysaccharide (LPS) 3 days later to induce clinical symptoms (e.g. swelling of hind paw and digits) (Figure 3).

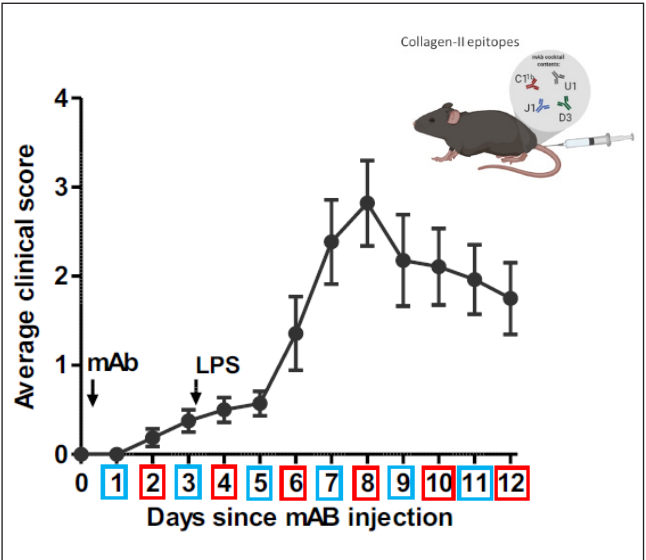


Figure 3. CAIA induction.

Here are examples of clinical scores observed in the paradigm (Figure 4) adapted from Coppard *et al* 2019.⁴

Figure 5 shows the development of mechanical sensitivity caused by collagen antibodies. i.e., model for mechanical pain caused by rheumatoid arthritis. The black line demonstrates clinical score (swelling of hind paw and digits), showing that increased inflammation is accompanied by decreased mechanical thresholds.

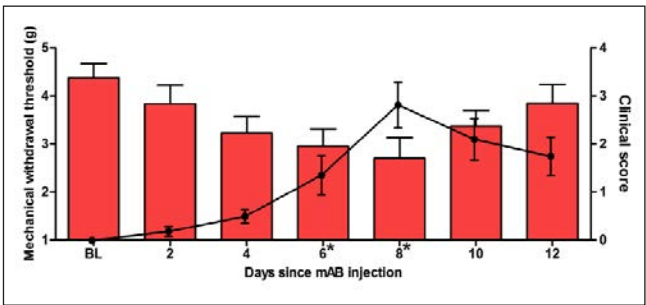


Figure 5. Development of mechanical sensitivity.

Development of cold pain (Figure 6) caused by rheumatoid arthritis in this model. The black line demonstrates how cold sensitivities compare to inflammation.

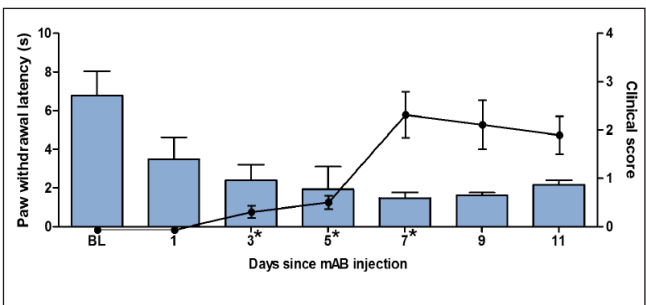


Figure 6. Development of cold pain.

Correlation of mechanical thresholds with ankle width (Figure 7). Demonstrates that ankle width (often used as a surrogate for pain) has poor/no correlation with actual mechanical pain which is measured in red.

Correlation of cold sensitivities with ankle width which similarly demonstrates that ankle width has little/no correlation with actual cold pain thresholds (Figure 8).

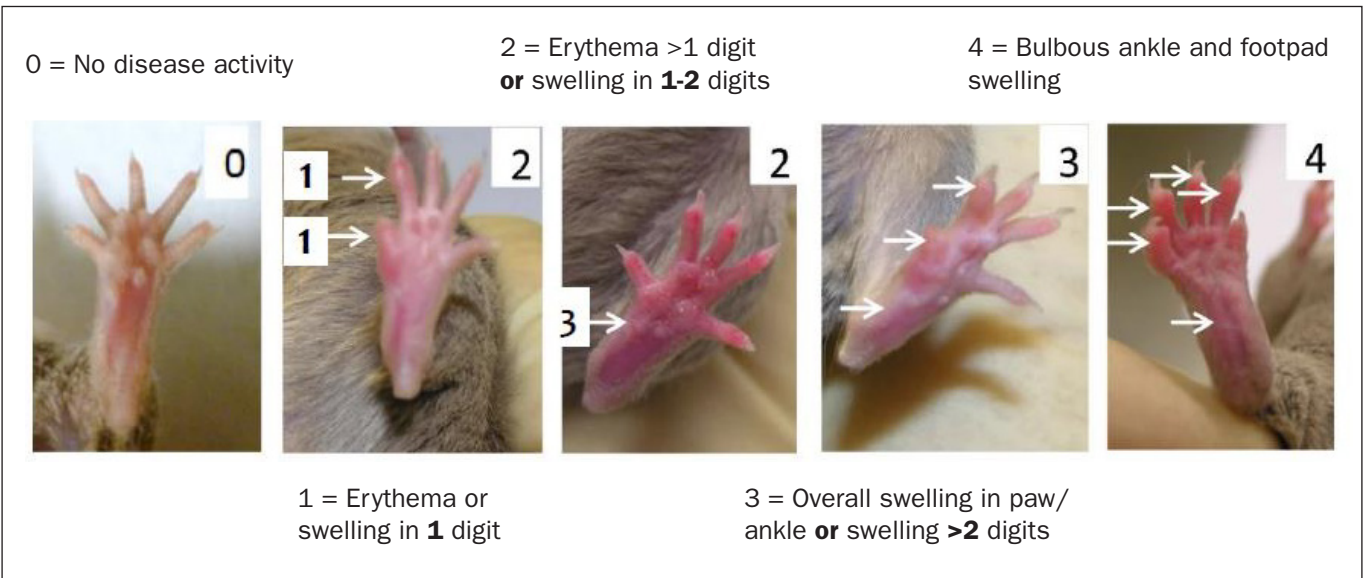


Figure 4. Clinical scoring.

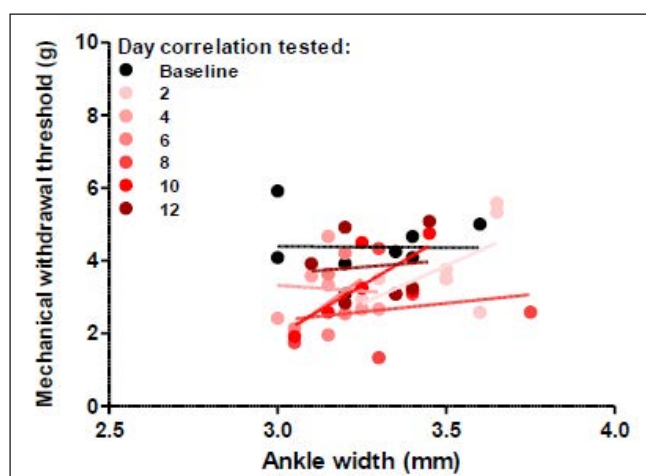


Figure 7. Correlation of mechanical thresholds.

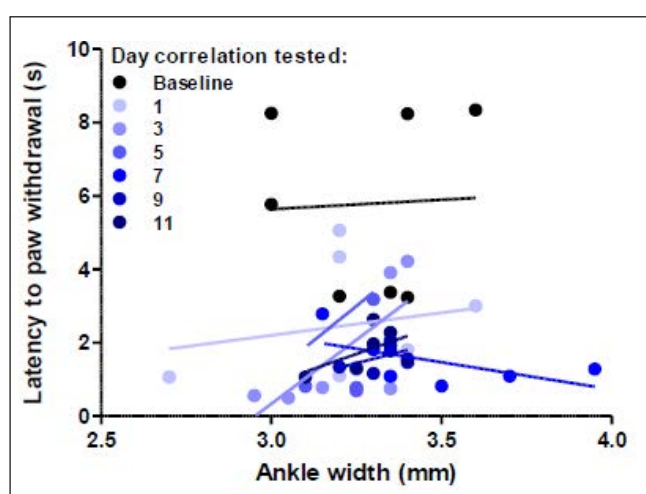


Figure 8. Correlation of cold sensitivities.

Figure 9 shows the correlation values for mechanical pain.

Mechanical withdrawal threshold correlation to ankle width:

Day tested	Pearson's r value	P value	Significant?
Baseline	-0.015 (-0.71–0.70)	0.97	Not significant
2	0.59 (-0.20 – 0.91)	0.13	Not significant
4	-0.08 (-0.84 – 0.78)	0.88	Not significant
6	0.51 (-0.51 – 0.93)	0.3	Not significant
8	0.20 (-0.73 – 0.87)	0.7	Not significant
10	0.75 (-0.15 – 0.97)	0.08	Not significant
12	0.10 (-0.77 – 0.84)	0.85	Not significant

Figure 9. Mechanical pain.

The correlation found to be significant was on day 5 (Figure 10).

Cold hypersensitivity correlation to ankle width:

Day tested	Pearson's r value	P value	Significant?
Baseline	0.046 (-0.73–0.77)	0.92	Not significant
1	0.20 (-0.65 – 0.83)	0.66	Not significant
3	-0.035 (-0.77 – 0.74)	0.94	Not significant
5	0.86 (0.16 – 0.98)	0.028	Significant*
7	-0.6 (-0.95 – 0.41)	0.21	Not significant
9	0.47 (-0.55 – 0.93)	0.35	Not significant
11	0.56 (-0.46 – 0.94)	0.25	Not significant

Figure 10. Correlation values for cold pain.

- Pain in collagen antibody induced arthritis. (A) Time course of development of mechanical hyperalgesia. (B and Bi) Correlations between ankle width and mechanical withdrawal thresholds on each day of testing.
- (C) Time course of development of cold hypersensitivity. (D and Di) Correlations between ankle width and paw withdrawal latency from cold water on each day of testing.

In each case * indicates $P < 0.05$ from baseline.

Collagen antibody treated mice were scored as 'sad' as evident by their quiescence, hunched posture and lameness. (Figure 11.) Mice which received morphine at 3mg/kg displayed significantly fewer observations on all qualitative parameters.

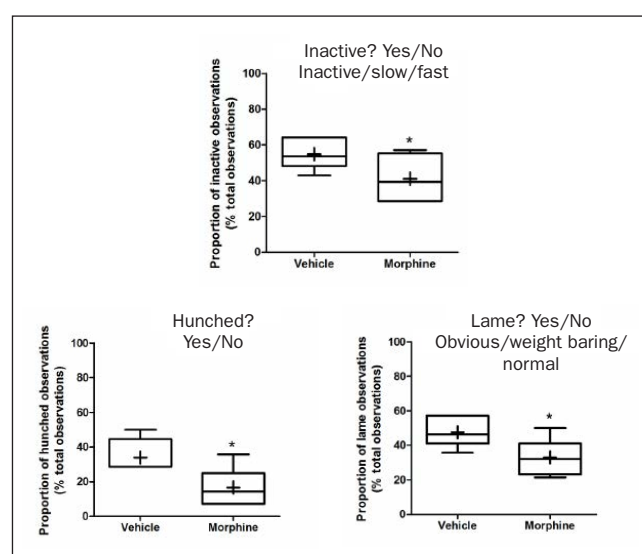


Figure 11. Affective measures of analgesia.

The scoring was performed by trained Animal Technicians who participated in the daily welfare checks and were blind to the treatment outcome.

This demonstrates that qualitative assessment of pain and analgesia is a better outcome compared to a single surrogate of hind paw width.

Additional observations that were scored included grooming status, ear position and piloerection (data not shown).

Conclusion

- Footpad widths poorly reflect pain experienced in arthritis models.
- Observing the posture and activity of an animal might better indicate whether they are in pain.
- Qualitative and semi-quantitative measures of affective pain can be useful in determining when an animal should receive analgesia.
- Further qualitative refinements should be added to identify better humane endpoints.

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Samuel Singleton assessed pain.

References

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