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A simple score for assessing bone erosion in rodent arthritic paws visualised by micro focal Computer Tomography X-ray.

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KINACEPT

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INTRODUCTION

The EU KINACEPT programme is investigating mechanisms behind joint erosion in rheumatoid arthritis as part of its Drug Discovery program. Rodent arthritis models result in joint destruction that traditionally requires observer scoring of histological sections. Micro-CT systems linked with sophisticated software can now visualise entire paws in three dimensions. However, even now, quantitative analysis of the multiple joints found in the paw remains difficult and labour intensive. Algorithmic calculations can be used to calculate indices such as bone surface roughness (Silva et al., 2006), bone size (Silva et al, 2004, Proulx et al, 2007), and bone cortical volume (Barck et al., 2004). Whilst such systems are being developed to improve experimental throughput, we have devised a visual score for the quantitative determination of bone erosion in whole paws, termed the S&M (Seed & Mancini) score.

METHODS

Collagen-induced arthritis was induced in male dba1 mice. Arthritis was scored arthritis, with each affected digit and ankle/wrist = 1, max = 22, and hind paw inflammation plethysmometry. Paws were taken at 32-33 days and fixed in formal saline. Micro-CT images were acquired using a Siemens Microcat II instrument, scan duration 30 minutes. Images were reconstructed with 768 z slices each having 512 x 512 pixel resolution (32 mm x 32 mm). For isosurface plots, density thresholds were set by reference to intact mouse metatarsal bone (AMIRA). The erosion of the metatarsophalangeal (MTP) joints was scored as follows:

0 = Anatomically Normal;

1 = Point erosion metatarsal;

2 = as 1, + elongated erosion;

3 = as 2, + complete penetration;

4= as 3 + elongated penetrative eroded metatarsal, erosion in proximal phalangeal bone;

5 = as 4 + complete penetrative erosion of proximal phalangeal bone;

6 = as 5, + MTP joint destroyed.

The scores for each of four digits are summed to give a maximum of 24.

Figure 1. Example of the scoring system for four digits of paws from collagen induced arthritic mice – full paws
Micro-CT images were acquired using a Siemens Microcat II instrument, scan duration 30 minutes. Images were reconstructed with 768 z slices each having 512 x 512 pixel resolution (32 mm x 32 mm). For isosurface plots, density thresholds were set by reference to intact mouse metatarsal bone (AMIRA).

Figure 2. Examples of the MTP joints with associated scores.

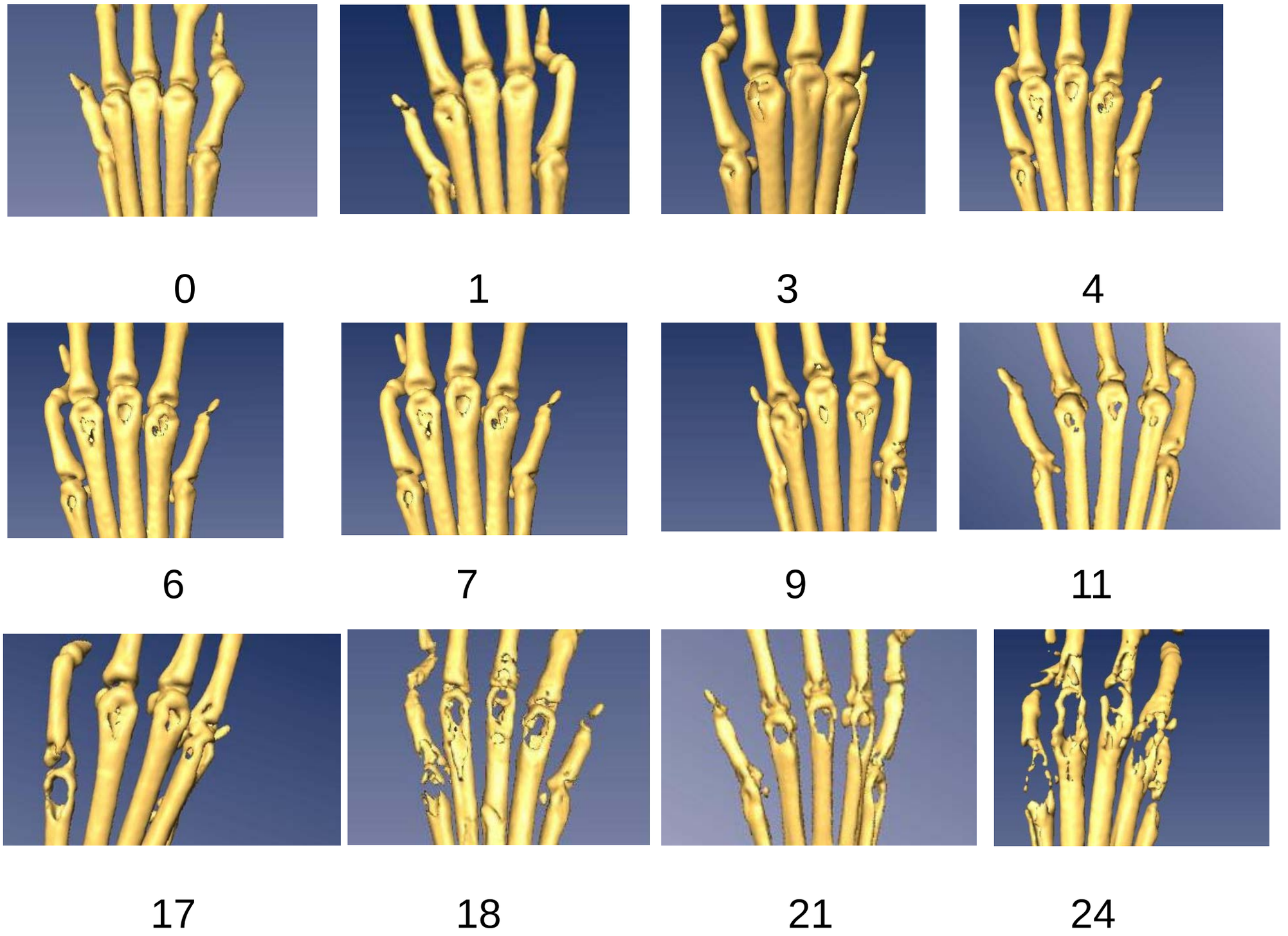
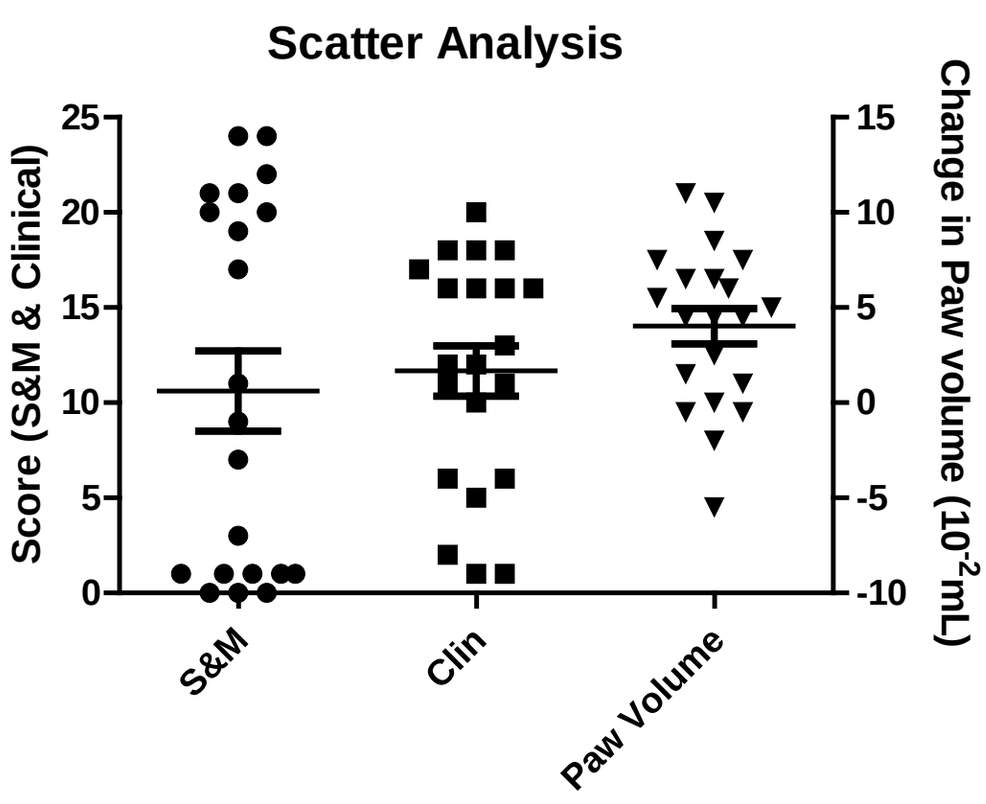


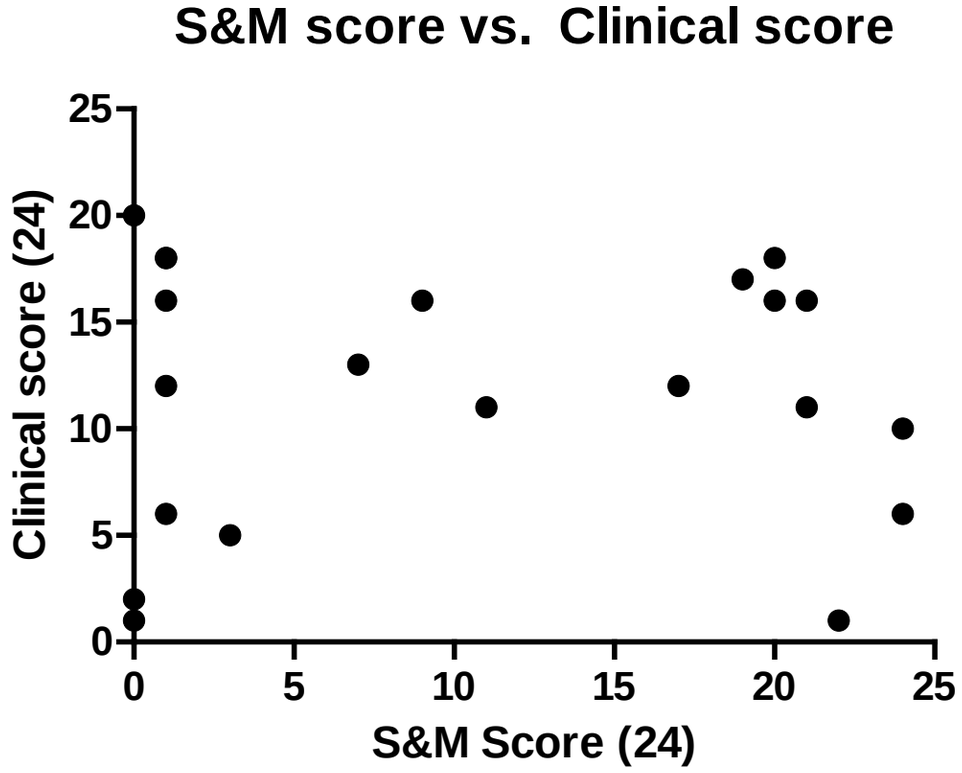
Figure 3 Gaussian distribution Analysis. Mice responded to an almost all-or-nothing MTP erosive response, with two populations ($p<0.0001$). The Clinical Scores appeared to diverge, however this was not significant. The increase in paw volume was normally distributed.



D'Agostino & Pearson omnibus normality test

	S&M	Clinical	Paw Volume
K2	19.46	2.650	0.7193
P value	< 0.0001	0.2659	0.6979
Passed normality test (alpha=0.05)?	No	Yes	Yes
P value summary	***	ns	ns
Sum	223.0	245.0	84.50

Figure 4: Correlation between the MTP S&M erosion score and clinical score. Spearman correlation coefficient r=-0.106. There is no correlation between the two indices. This was largely due to three mice with high or low clinical scores possessing low and high erosion scores respectively



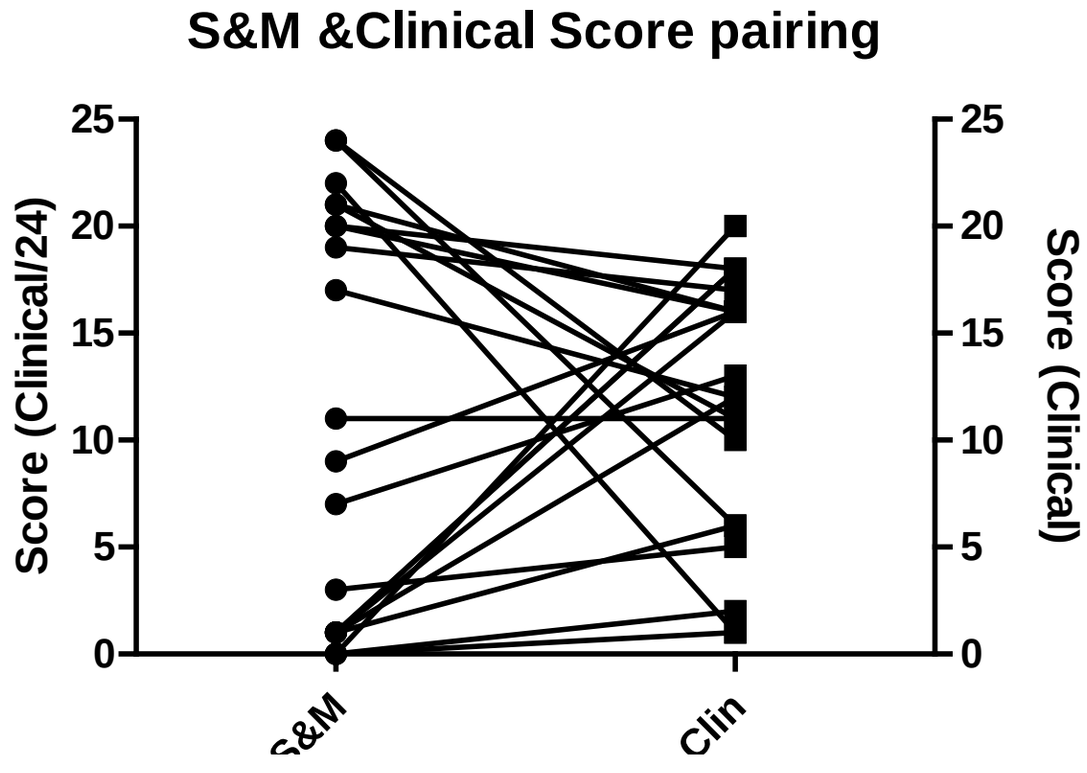
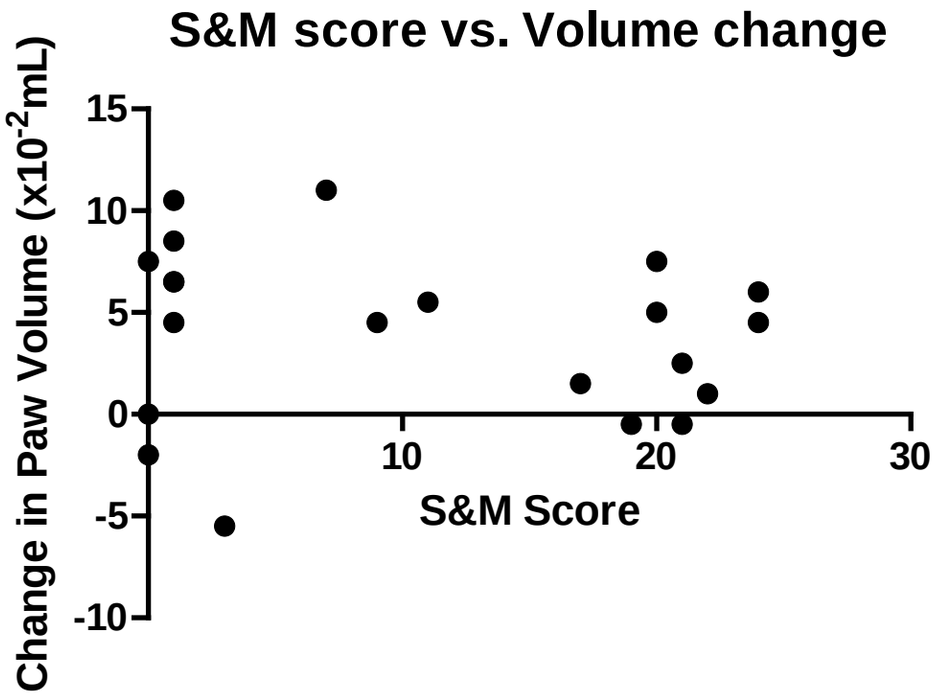
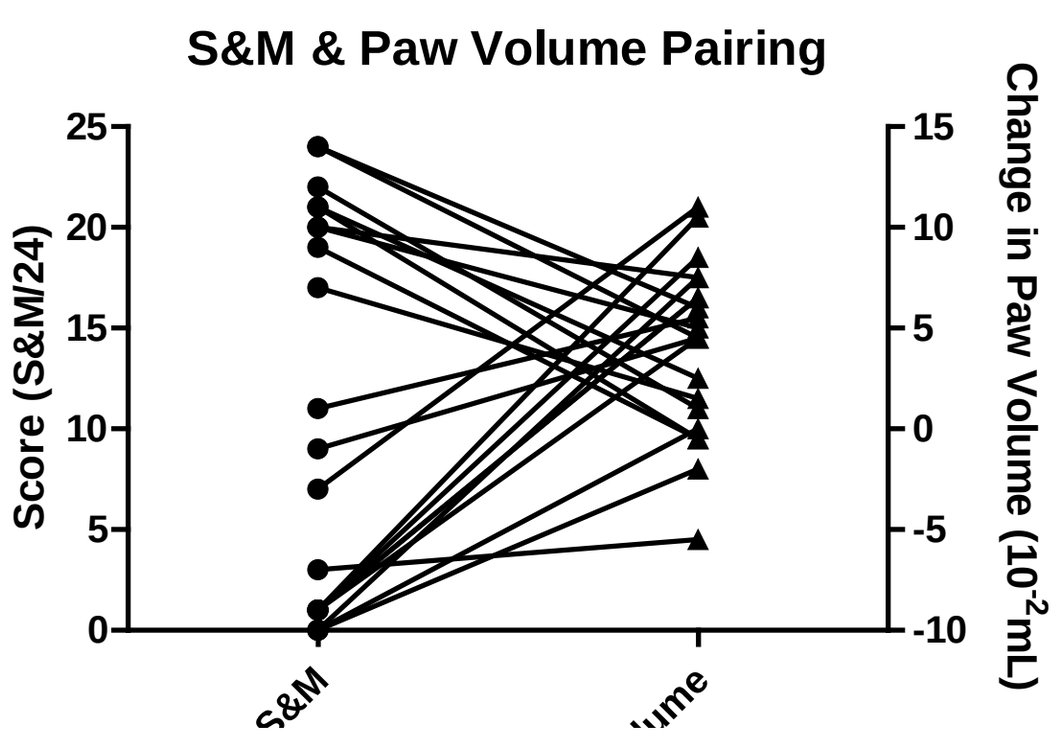


Figure 5: Correlation between the MTP S&M erosion score and change in paw volume. Spearman correlation coefficient r=-0.161. There is no correlation between the two indices. 5 mice with zero score had erosions amongst the top 50 centile.





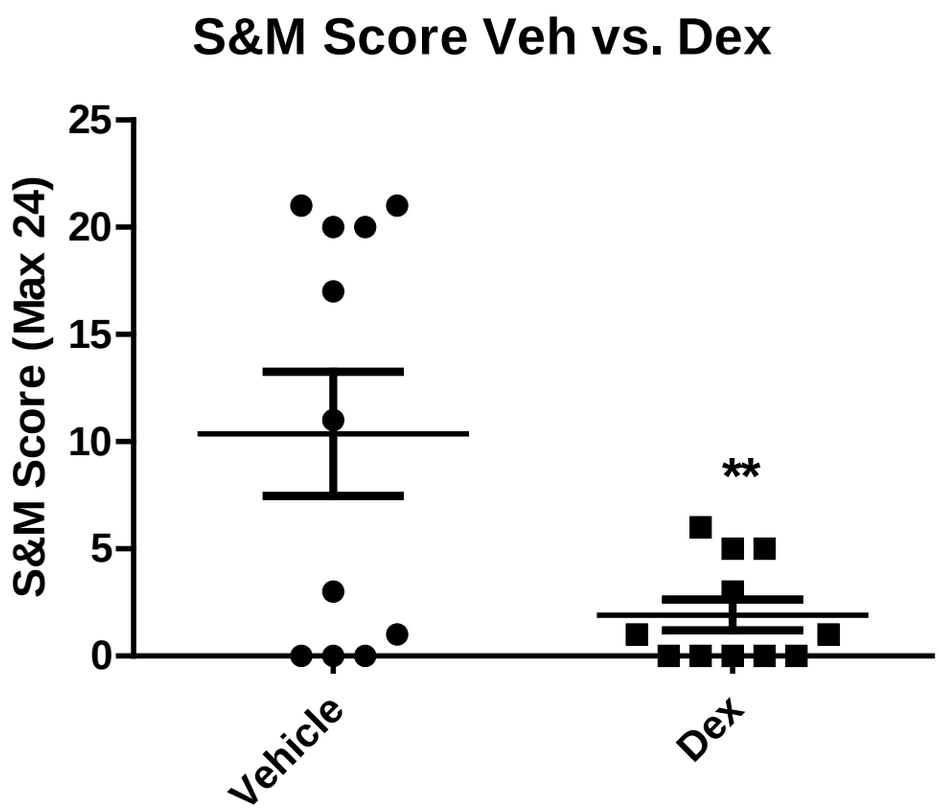


Figure 6: The inhibition of MTP erosion by dexamethasone (0.1mg/kg dosed therapeutically from day 21 [boost]) with representative score for median and IQR. **=p<0.01 Fishers exact test vs. vehicle controls (water)

Vehicle

Median = 11

25% IQR = 0

75% IQR = 20

Dexamethasone

Median = 1

25% IQR = 0

75% IQR = 5

CONCLUSIONS:

We have described a simple scoring method that quantifies the erosion of MTP joints from collagen arthritic mice as visualized by micro-CT X-ray isosurface plots. At termination (Days 33 & 35) half of the mice have significant and severe erosion, whilst the remainder are relatively erosion free, despite dba mice being an inbred strain. The level of erosion does not correlate with the degree of disease as assessed by clinical scoring or paw inflammation. Paired analyses reveals extremes in subsets with inverted relationships, i.e. high erosion:low inflammation and low erosion:high inflammation. The score detects significant inhibition on therapeutic treatment with dexamethasone. , their erosive disease splits between two populations.

Barck K.H. *et al.*, (2004) *Arthritis Rheum.* 50, 3377-3386
Proulx S.T. *et al.*, (2007) *Arthritis Rheum.* 56, 4024-4037
Silva M.D. *et al.*, (2004) *Mol. Imaging* 3, 312-8
Silva M.D. *et al.*, (2006) *Mol. Imaging* 5, 475-484

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