Assessment of systemic and local bone loss in the model of antigen-induced arthritis

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INTRODUCTION & AIMS

- rheumatoid arthritis (RA) is a chronic autoimmune arthritis
- bone and cartilage destruction in the inflammed joints, as well as a systemic osteopenia are present
- systemic vs. local bone loss – separate mechanisms and a different clinical significance
- antigen-induced arthritis (AIA) is considered to have few or no systemic effects

However, as the immunization protocol induces a systemic immune response, we suspected that it may also affect bone mass at the systemic level.

OBJECTIVE: to determine the extent of local bone destruction and generalized osteopenia in AIA

- µCT: assess trabecular bone volume in the second lumbar vertebrae (L2), femoral epiphyses and metaphyses
- flow cytometry: determine the proportion of myeloid (CD11b+Gr1+) cells in the spleen, femoral bone marrow and knee joints

MATERIALS & METHODS

8-10 week old male C57BL/6 mice, divided into 3 groups:
1. non-immunized group (NI)
2. immunized control group (CTRL)
3. arthritis group (AIA)

day 0 (primary immunization) - **NI**: PBS s.c.; **CTRL** & **AIA**: mBSA in CFA s.c.
day 7 ("booster") - **NI**: PBS s.c.; **CTRL** & **AIA**: mBSA in CFA s.c.
day 21 (induction of arthritis) - **NI**: PBS i.a.; **CTRL**: PBS i.a.; **AIA**: mBSA in PBS i.a.
day 31 (assessment)

RESULTS

Statistically significant decreases in bone volume were found in the L2 and metaphyses of CTRL and AIA mice, in comparison to NI mice ($p<0.05$, Kruskal-Wallis test). On the other hand, the epiphyseal trabecular bone volume was significantly lower in AIA mice in comparison to CTRL and NI mice ($p<0.05$, Kruskal-Wallis test). In both immunized groups there was a significant increase in the proportion of myeloid cells in the spleen, bone marrow and knee joints, in comparison to the control group.

CONCLUSIONS

The AIA model of arthritis induces systemic osteopenia, which should be taken into account, in particular when addressing the local osteodestructive changes. Metaphyseal bone volume better reflects the systemic features of the model, whereas epiphyseal trabecular bone volume is a more reliable indicator of the local ostedestructive changes.

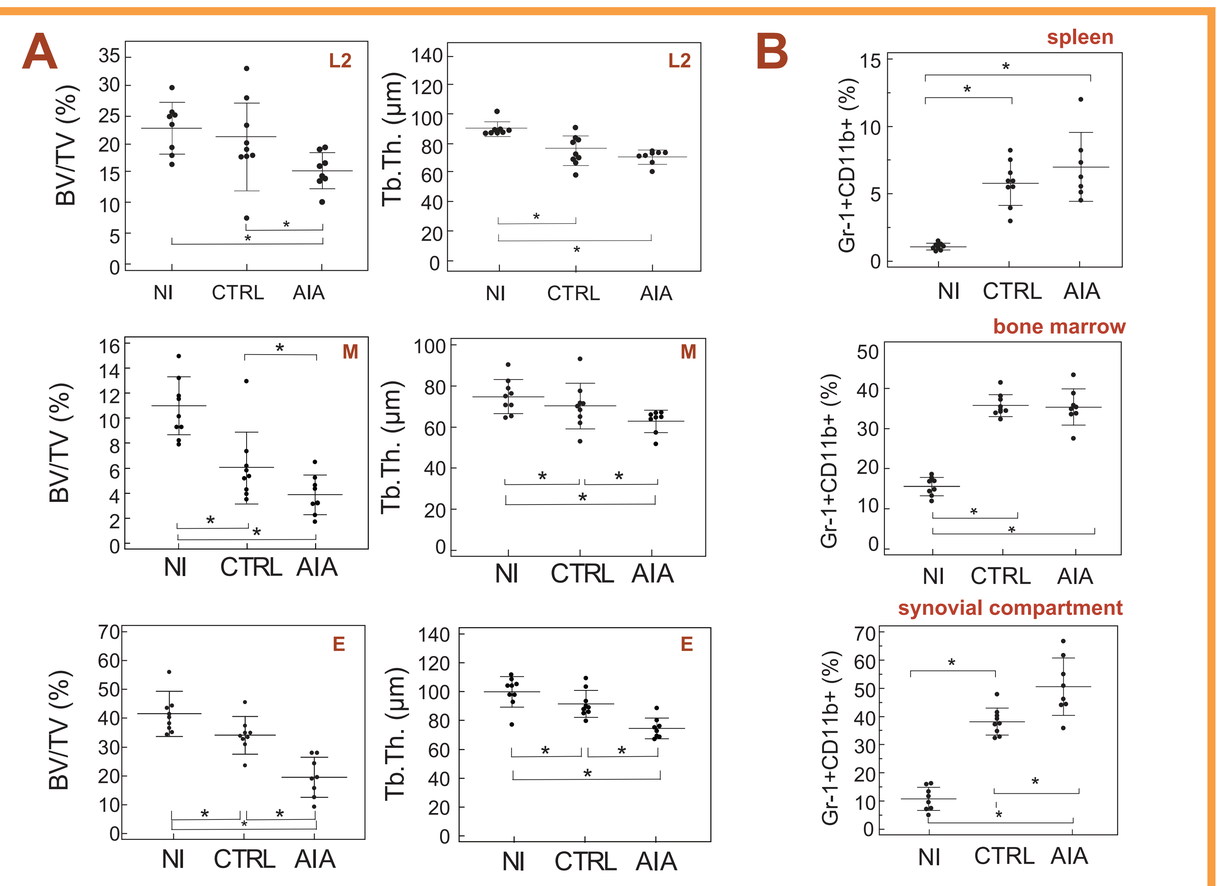

Figure 1. **A.** Micro-CT analysis of trabecular bone volume (BV/TV) and trabecular thickness (Tb.Th.) in second lumbar vertebrae (L2), femoral metaphyses (M) and epiphyses (E) from non-immunized mice (NI), immunized mice injected i.a. with PBS (CTRL), and mice with arthritis (AIA). L2 bone volume was significantly lower in AIA group in comparison with other groups. Although bone volume loss was most significant in femoral metaphyses, most of it can be atributed to immunization (47%), while arthritis caused an additional 19% loss. On the other hand, immunization had a weaker effect on femoral epiphyses, where it attributed to only 10% of bone volume loss, while AIA group had an additional 35% of bone volume loss. **B.** Significantly higher proportions of myeloid cells, marked by Gr1-PE and CD11b-PECy7 antibodies, were found in AIA and CTRL groups in comparison to NI group. Moreover, there was an additional increase of the myeloid cell proportion seen in arthritis (AIA) group in the synovial compartment in comparison to immunized controls (CTRL).

*statistically significant difference, $p<0.05$, Kruskal-Wallis test

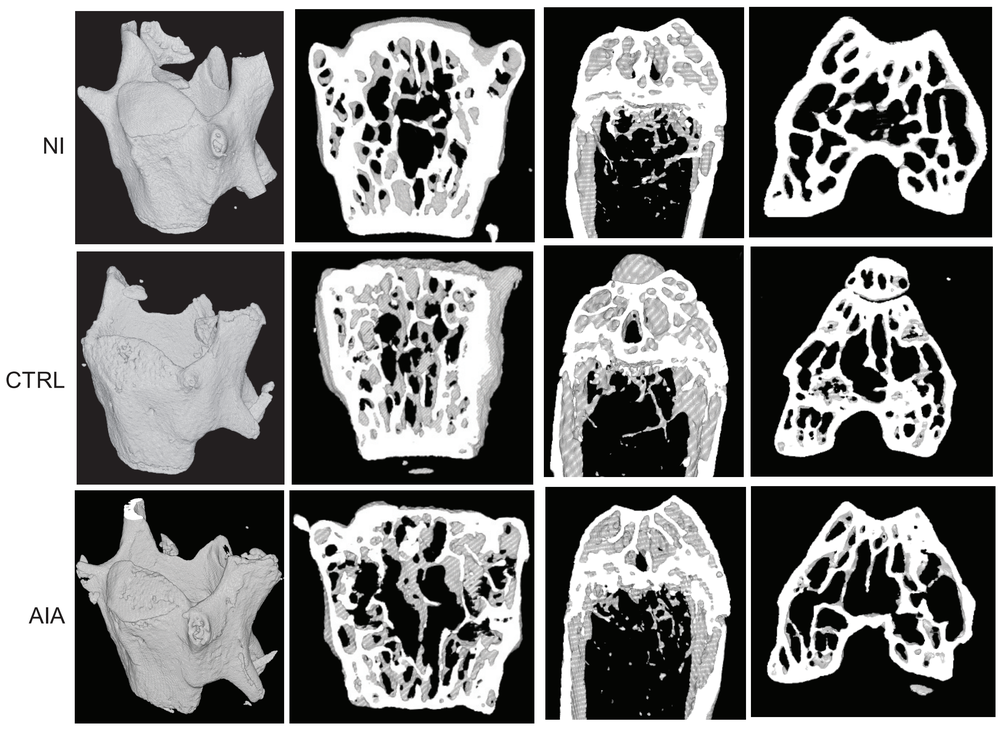

Figure 2. Representative 3D models of the second lumbar vertebrae (L2) (**A** ,**B**), femoral metaphyses (**C**) and epyphises (**D**) from micro-CT reconstruction images from non-immunized mice (NI), immunized mice injected i.a. with PBS (CTRL), and mice with arthritis (AIA). Multiple bone erosions are visible on L2 vertebrae from CTRL and AIA groups, while there are no similar changes in NI group (**B**). Bone loss and/or metaphyseal and epyphiseal trabecular thinning is seen in CTRL and AIA groups in comparison to non-immunized group (**C**,**D**).

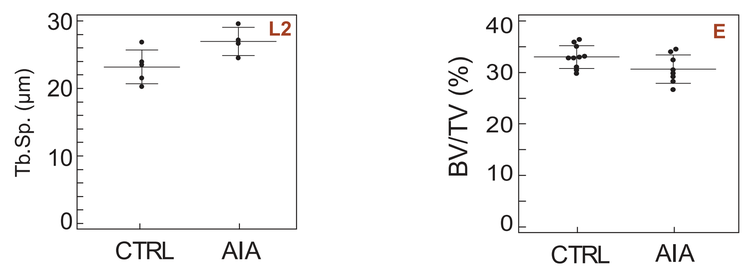

Figure 3. In order to minimalise the effect of immunization, we conducted a short AIA protocol which consisted of intraarticular arthritis (i.a.) induction 7 days after primary immunization. Mice sacrifice and µCT assessment were conducted 7 days after i.a. induction (14 days after the start of the experiment). Trabecular seperation (Tb.Sp.) in the second lumbar vertebrae (L2) and trabecular bone volume (BV/TV) in the femoral epiphyses (E) only slightly differed from controls ($p=0.0504$ and $p=0.0506$ respectively), while other parameters were not significantly different. Upon macroscopic examination, there were no clear signs of arthritis in either group.

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