Bone Histomorphometry 101

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Role of Bone Histomorphometry

• histological assessment of bone phenotypes
• directly visualize bone cells in relation to tissue, at baseline or in disease models
• allows comparisons between groups even when differences are not visually “obvious”
• most useful when interpreted in the context of other data such as structural analysis (microCT, DEXA), serum markers of bone turnover, and/or disease scoring
Choice of controls

- littermates are best
- age/sex matched
- same strain from different suppliers are not the same in bone mass, so beware of buying controls

Mouse strains vary a lot
C57/Bl6 may not be the “best” since bone mass is relatively low, but it is what we often have to use
A quick editorial

• Although histomorphometry gives you numbers with standard deviation, p values, etc, the parameters that you measure are not black and white, and thus are somewhat subjective
• This lack of objectivity can be especially problematic if the one measuring is biased, either consciously or subconsciously
• Therefore, you should always be blinded
  – have a labmate relabel your slides and keep the key
  – have a labmate take photos for you code them
Choice of skeletal site


borrowed from Natalie Sims
Femur vs tibia
More choices

• Plastic (methyImethacrylate) vs. paraffin
  – plastic is used for mineralized bone, paraffin requires demineralization (eg. with EDTA)
  – bone formation (calcein labels) can only be analyzed in plastic sections
  – stains that differentiate mineralized bone from osteoid (vonKossa, trichrome) can only be done on plastics
  – BUT, plastic takes longer and is more expensive
Which stains should I order?

- H&E – good for seeing bone vs cartilage (growth plate) and seeing OBs, but not OCs, good to see tumor or inflammation
Which stains should I order?

- TRAP – highlights OCs, still OK for bone vs cartilage, sometimes OBs hard to see
Which stain should I order?

- vonKossa – shows osteoid vs bone, not good for cellular detail
- trichrome (Masson or Goldner) – shows osteoid, and better for cellular detail, but OCs may be hard to ID
Which stains should I order?

- safronin O and toluidine blue – good for cartilage
Standard nomenclature


• Some aspects of this system are confusing
• We measure parameters on 2 dimensional slides, but we talk about volumes and surfaces
  – in microCT, BV/TV is a volumetric measurement
  – in histomorphometry BV/TV = bone area/tissue area
  – some formulas are used in Bioquant for converting perimeters to surfaces
Define your region of interest
Primary Measurements
(things you measure directly)

- **Area (volume)** – outlines a closed space
  - bone volume (BV or B.Ar) = amount of bone in your ROI
  - tissue volume (TV or T.Ar) = amount of tissue (bone+ marrow) in your ROI
  - osteoid volume (OV or O.Ar)

- **Length - lines**
  - bone surface (BS or B.Pm)
  - osteoblast surface (Ob.S or Ob.Pm)
  - single labeled surface (sLS or sL.Pm)

- **Distance – between 2 lines**
  - osteoid thickness (O.Th or O.Wi)
  - interlabel distance (Ir.L.Th)

- **Number - dots**
  - osteoblast number (N.Ob)
  - osteoclast number (N.Oc)
A note on eroded surface (ES)

- eroded surface is the surface of the lacuna generated by an active OC

In mice, however, it is often hard to see nice pits since the OCs seem to sit on a flat bone surface. I consider ES in mice to be very subjective, compared to Oc.S (which still involves some judgment calls).
Derived structural indices (things that get calculated from your primary measurements)

— trabecular number $Tb. N = (BV/TV)/Tb. Th$
— trabecular separation $Tb. Sp = (1/Tb. N) - Tb. Th$

• These, as well as $BV/TV$, are better assessed by microCT
• However, in order to get values for cell counts and surfaces, you always have to measure the bone area and bone surface on your sections
Dynamic histomorphometry

- measurement of bone formation parameters
- mice are given 2 doses of label several days apart, by IP injection
- calcein (green) and/or alizarin red (red) incorporate into newly calcifying bone
- interval depends on age of mice
  - 2-4 week mice, 3-4d
  - 6-12 week mice, 5-7d
  - older mice, 7-10d
- easier to measure in calvaria than trabecular bone
Derived kinetic indices

• Mineralizing surface  \( MS = (dLS + sLS/2)/BS \)
  – the extent of bone surface actively mineralizing

• Mineral apposition rate  \( MAR = \frac{Ir.L.Th}{Ir.L.t} \)
  – distance between the labels divided by time between labels

• Bone formation rate  \( BFR = MAR \times \left( \frac{MS}{BS} \right) \)
  – multiplies the MAR by the fraction of bone surface that is labeled
MAR vs BFR

**MAR** = $\text{Ir.L.Th}/\text{Ir.L.t}$

- **Ir.L.t** = time between injection of labels

MAR is the rate at which OBs are making matrix, which calcifies at a constant rate and incorporates the labels. Thus, it measures the average activity of the OBs in your section.

**BFR** = **MAR** * (MS/BS)

BFR takes into account how much of the bone surface is actively mineralizing, which depends on the number of OBs that are active. It multiplies the average work of each OB by the fraction of bone surface with active OBs.
Static vs dynamic parameters

ie. OB counts vs calcein labels

• Counting OBs based on morphology is difficult, as some are more “classic” than others

• It is possible to do an alkaline phosphatase stain on plastic sections, but as you have seen with OC counts, that will not be perfect either

• Most people consider the dynamic indices to be a better reflection of OBs than static counts, since it accounts for “activity per OB” and how “number of OBs” (active surface)
Histomorphometry in Inflammatory Arthritis

WT

NIK\(^{-/-}\)

![Histological images comparing WT and NIK\(^{-/-}\) conditions](image)

![Graphs showing inflammation and bone erosion scores](image)

![Graphs showing osteoclasts (OC) per mm](image)

Aya JCI 115: 1848
Antigen-induced arthritis

quantify area with inflammation
count OCs on bone surface
Antigen-induced arthritis

quantitate extent of safO staining on articular surface
Bone metastasis

quantitate tumor area/tissue area (tumor volume/tissue volume)
Analysis of fracture model looking at vessels

Silva group

Calcif Tissue Int (2007) 80:391–399