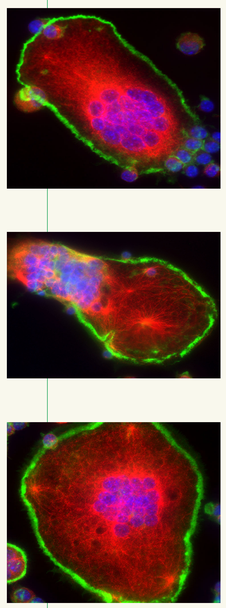
The *In vitro* Osteoclast Differentiation in Arthritis (IODA) project:
Osteoclastogenesis as a marker of presence and activity of disease in Rheumatoid Arthritis and Osteoarthritis

A. de Brum-Fernandes[1], G. Boire[1], J. Dixon[2], M. Durand[1], R. Harrison[3], S. Komarova[4], L. Kurgan[5], M. Manolson[3], M. Osama[4], R. Rak[5], M. Roy[1], S. Sims[2]

[1]Division of Rheumatology, Department of Medicine, Faculty of Medicine and Health Sciences, Université de Sherbrooke; [2]Department of Physiology and Pharmacology, University of Western Ontario; [3]Dental Research Institute, Faculty of Dentistry, University of Toronto; [4]Faculty of Dentistry, McGill University; [5]Department of Electrical and Computer Engineering, University of Alberta.



Introduction

Osteoclasts play an important role in the pathophysiology of rheumatoid arthritis (RA) and osteoarthritis (OA). We hypothesize that *in vitro* osteoclastogenesis and the activity of these osteoclasts correlate with disease presence in RA and OA patients and with disease activity in RA. We previously showed that patients with RA have higher bone resorption rates and that patients with inactive RA have higher osteoclastogenesis rate than the group with active RA or controls. In this work we extend the analysis of the RA cohort and study the OA cohort.

Objectives

To determine whether osteoclast function, osteoclastogenesis, and other related factors are related to:

- the presence of RA (by contrasting RA patients and controls)
- the activity of RA (by contrasting patients with active and inactive RA)
- the presence of OA (by contrasting OA patients and controls)

Methods

Subjects

140 patients with RA and 112 with OA were recruited from the outpatient clinic at the Division of Rheumatology, Centre hospitalier universitaire de Sherbrooke. Sixty self-reported healthy controls were recruited from local population.

Osteoclastogenesis

Peripheral blood mononuclear cells were separated from blood by Ficoll gradient and the number of CD14+ cells was determined by flow cytometry. The cells were cultured in the presence of RANKL and M-CSF and the number of OCs was evaluated after 21 days. Bone resorption was quantified on cortical bone slices stained with toluidine blue. OC apoptosis was evaluated by colorimetric assay (TACSTM TdT Blue Label kit).

Data analysis

We developed human-readable prediction models (*alternating decision trees*), one for each objective, capable of discriminating patients with regard to those objectives. The advantage of using descriptive methods lies in the simplicity of the model, which is human-interpretable, as opposed to other methods that create a mathematical or probabilistic model. The models (see Figures 1 and 3) were used to extract potential markers that were confirmed using standard statistical methods (see Figure 4).

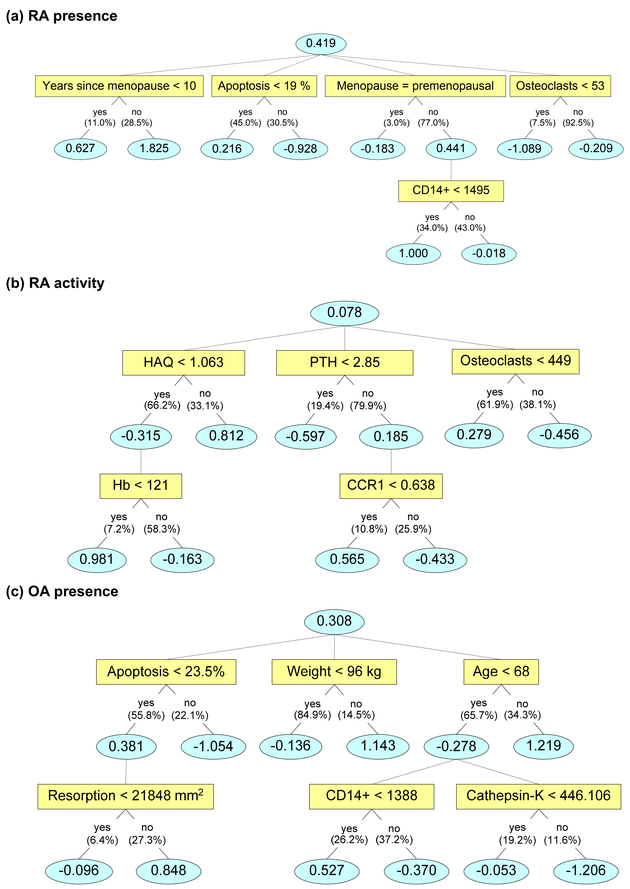
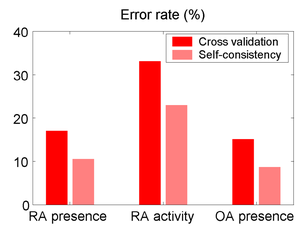


Figure 1. Alternating decision tree models for the three objectives.

Descending from the top node downwards along the branches, if the sum of scores (numbers in blue ovals) satisfying the conditions (shown in associated yellow rectangles) is positive then the prediction is positive (the disease is present in case of (a) and (c) or the disease is active in case of (b)). Otherwise (negative sum), the prediction is negative. The bigger the sum (in either direction), the stronger the confidence associated with the prediction. Values in parentheses (right below "yes" and "no") is the percentage of patients satisfying (or not) a given condition.



- **Cross validation** indicates the ability of a model to classify unseen data
- **Self consistency** shows how well the model describes the data it was built from

Figure 2. Prediction error rates for the three models

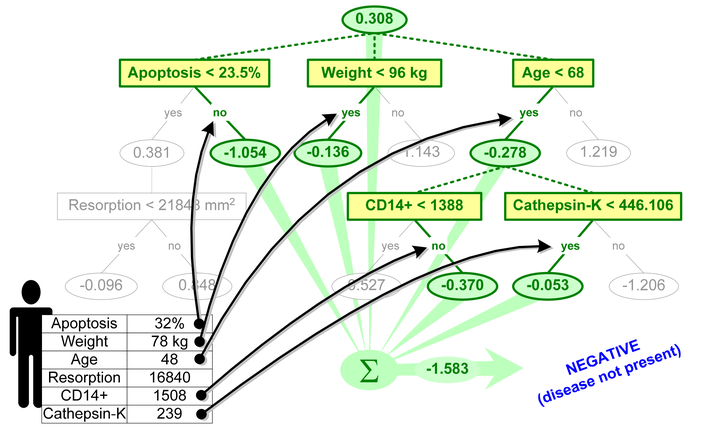


Figure 3 Using OA presence model to predict whether a patient has OA.

The patient's set of features is compared against the model. The sum of branches satisfying the conditions is negative, which indicates that the patient has no OA.

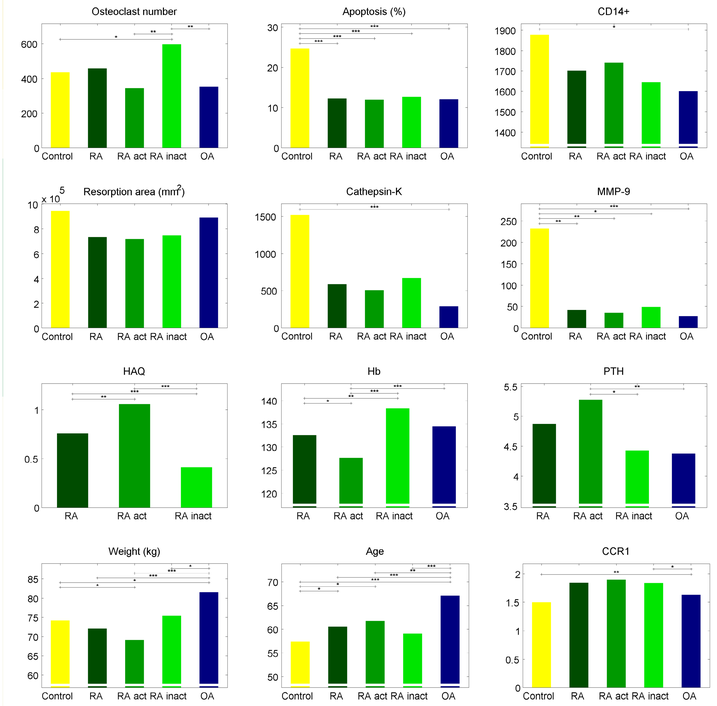


Figure 4. Statistical analysis showing the difference in mean between groups for the most significant markers.

* P value < 0.05, ** P value < 0.01, *** P value < 0.001; T-test and Mann-Whitney test were used for normal and non-parametric variables, respectively; Shapiro-Wilk test was used to verify normality.

Results

The models were built upon patients' features including demographics, clinical observations, lab tests, and osteoclastogenic information. Different feature sets were used for different objectives. For instance, there are more data available for the RA activity study, compared to the RA presence study, due to a more thorough clinical and lab analysis performed on patients with RA, when compared with controls included in the RA presence study.

RA presence study

- Higher Apoptosis (above 19) is associated with the absence of the disease (31% of patients).
- The lower the number of osteoclasts, the lower the chances of having the disease.
- Lower CD14+ (below 1495) is associated with the presence of the disease (34% of patients).
- Patients with ten years since menopause are likely to have RA (29% of all patients); however, this may represent a selection bias as RA patients are chronic patients seen over a long time while OA and controls are seen once.

RA activity study

- Lower HAQ (below 1.063) together with higher Hb (above 121) are strongly associated with inactive disease (58% of patients).
- Lower PTH (below 2.85) is associated with inactive disease (19% of patients).
- Higer number of osteoclasts (above 449) is associated with inactive disease (38% of patients).
- Higher PTH together with lower CCR1 are associated with active disease (11% of patients).

OA presence study

- Higher Apoptosis (above 24%) is strongly associated with the presence of the disease (22% patients).
- For patients younger than 68 the higher Cathepsin-K the lower the chances of the disease; whereas lower CD14+ (below 1388) is associated with the presence of the disease.
- Lower Apoptosis (below 24%) together with higher Resorption are strongly associated with the presence of OA (27% of patients).
- Patients older than 68 (34% of all patients) as well as patients with high weight (above 96 kg) (15% of patients) are likely to have OA; however, this may represent a selection bias.

Conclusions

Overall, the results indicate that parameters important to osteoclast biology correlate with the presence of OA and RA and with the activity of RA.

Acknowledgement

Pfizer
CANADIAN ARTHRITIS NETWORK | LE RÉSEAU CANADIEN DE L'ARTHRITE
CIHR IRSC Canadian Institutes of Health Research | Instituts de recherche en santé du Canada
UNIVERSITÉ DE SHERBROOKE