Search Strategy, Assessment of Risk of Bias, and Statistical Methods

We did a systematic review and meta-analysis in accordance with the PRISMA (Preferred Reporting Items for Systematic reviews and Meta-analyses) guidelines. All randomized controlled trials (RCT) comparing bisphosphonates to controls (other bisphosphonates, placebo, or no drug) for the treatment of low bone mineral density (BMD) in HIV-infected adults were included. All doses and routes of administration were considered. Trials of a minimum duration of six months were included. Outcomes of interest were BMD changes measured by dual-energy X-ray absorptiometry (DXA) at the lumbar spine, femoral neck, and total hip, and adverse events.

Relevant RCTs were identified by electronic searching of the Cochrane Central Register of Controlled Trials (CENTRAL) in The Cochrane Library, MEDLINE, EMBASE, LILACS with no restrictions on the language and year of publication. The lists of references were manually searched to add any citations missed by the electronic searches. The conference databases Aegis and NLM gateway were searched for abstracts presented at scientific meetings and included if the information was available in the body of the abstract. We also hand searched conference proceedings from the CROI, International AIDS Conferences, and International AIDS Society (IAS) Conferences on HIV Pathogenesis, Treatment, and Prevention from 2005 to 2012. We searched the prospective trials registers ClinicalTrials.gov and Current Controlled Trials (www.controlled-trials.com/) to identify ongoing trials. Searches were conducted in September 2013. An update was made in July 2014, with the inclusion of the study presented at CROI 2014 by Negredo, et al.

Two authors (Pinzone and Nunnari) independently reviewed all studies identified by the search strategy. We resolved disagreements through consensus and discussion. The two authors used a pre-established data collection form to extract data on study characteristics (country, design, duration, year, sample size, source of funding), study population eligibility criteria (inclusion and exclusion), characteristics of participants (age, sex, viral load and CD4+ T-cell count, number of patients receiving protease inhibitors and tenofovir), type of intervention (dose of the bisphosphonate, frequency and mode of administration), type and dose of additional interventions and outcomes. Study authors were contacted in case of incomplete or unreported data.

Two authors (Pinzone and Nunnari) assessed the risk of bias of all included studies. The methodological quality and risk of bias in individual trials were assessed by means of the Cochrane Collaboration’s tool for assessing risk of bias in randomized trials. The assessment tool covers six bias risk domains: sequence generation; allocation concealment; blinding; handling of incomplete outcome data; selective outcome reporting; other potential sources of bias. We planned to investigate the potential for publication bias using a funnel plot, but the number of included studies was too small to enable meaningful analysis.

Data were analyzed using Review Manager 5.2.7. For dichotomous outcomes (i.e. number of participants with adverse events), we calculated risk ratios (RR) and 95% confidence intervals (CI).

For quantitative outcomes (i.e. BMD), we estimated the difference in mean percentage change from baseline in the bisphosphonate and control group. When BMD values were not presented in the manuscript text, an electronic caliper was used to extract the data from figures. For continuous outcomes, the effect estimates were combined using the fixed-effects model (also known as the weighted-average method). We examined statistical heterogeneity by the $I^2$ statistic and the Chi$^2$ test. The $I^2$ statistic describes the percentage of total variation across studies due to heterogeneity rather than chance. A value of 0% indicates no observed heterogeneity, larger values show increasing heterogeneity; substantial heterogeneity is considered to exist when $I^2$ is > 50%. For the Chi$^2$ test, we used a value of $p < 0.10$ to indicate the presence of statistically significant heterogeneity.
Risk of bias in the included studies (Fig. 1)

Sequence generation

Three trials used computer-generated randomization\(^3\). In five trials, the method of sequence generation was not adequately described\(^29,30-32,36\).

Allocation concealment

In one trial the allocation of participants was controlled by a central randomization unit\(^3\), in two trials randomization was coordinated by the pharmacy\(^30,34\), and in another trial allocation was controlled by staff that had no direct contact with the participants\(^3\). In four studies the adequacy of allocation concealment was unclear\(^29,31,32,36\).

Blinding

Of the five trials assessing alendronate versus placebo or no intervention, two were described as double-blind\(^3,36\) and were judged to be at low risk of bias; three RCTs\(^29-31\) were open-label studies and were judged to be at high risk of bias. Two trials assessing zoledronate versus placebo were double-blind\(^3,34\) and were judged to be at low risk of bias for this domain; one study was open-label\(^36\). The two extension studies published by Bolland, et al. were unblinded\(^40,41\).

Incomplete outcome data

Five trials performed an intention-to-treat (ITT) analysis\(^29,32-35\), two studies did not\(^30,31\). For the Negredo 2014 trial\(^3\), only an abstract was available and it is unclear whether an ITT analysis was performed. In the McComsey trial\(^3\), the number of dropouts was balanced across groups, but the reasons for dropouts were not specifically described. In the Rozenberg trial\(^3\), the reasons for dropouts in the two groups were not specifically described. Furthermore, 23% of patients did not complete the 96-week follow-up.

Selective outcome reporting

We were unable to obtain the protocol for three of the trials, which were rated as unclear for this domain\(^29-31\). In five cases the protocol of the study was available in a clinical trial registry\(^32,33,36\). However, in two cases\(^33,35\) the protocol was retrospectively registered so we had insufficient information to permit judgment. In one trial\(^3\) the protocol did not describe outcomes and was rated as unclear. Only one trial\(^34\) was judged to be at low risk of bias for selective outcome
reporting as all pre-specified outcomes were reported in the paper. As for the observational extension studies of Bolland, et al., the authors evaluated the effects of zoledronate administration one and four years after completion of the RCT. However, in the report published in 2008, only patients who accepted to continue the follow-up (77%) were included in the statistical analysis; 72% of patients enrolled in the RCT agreed to continue the follow-up and the results of the four-year observational study were published in 2012. In this paper the authors performed an ITT analysis and sensitivity analysis with imputation of missing data for BMD.

### Other potential sources of bias

In three RCTs, baseline imbalance in some participant characteristics was observed. In the McComsey trial, the median screening lumbar spine t-score was significantly lower in the alendronate arm in comparison with the placebo arm. In the Negredo 2005 trial, patients receiving alendronate were older and had lower dietary calcium intake compared to the control group. In the Huang trial, the baseline CD4+ T-cell count was significantly higher in recipients of zoledronate compared to placebo. The Huang trial was partly supported by Novartis Pharmaceuticals.