

Time to be positive about negative data?

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“With no positivity, there is no hope; with no negativity, there is no improvement.”
— Criss Jami, *Healology*

Karl Popper (1902-1994), one of the greatest philosophers of science of the 20th century, writes in *Conjectures and Refutations* (Harper & Row, 1963): “Criticism of our conjectures is of decisive importance: by bringing out our mistakes it makes us understand the difficulties of the problem which we are trying to solve. This is how we become better acquainted with our problem, and able to propose more mature solutions: the very refutation of a theory –that is, of any serious tentative solution to our problem- is always a step forward that takes us nearer to the truth. And this is how we can learn from our mistakes”.

This special issue of *Osteoarthritis and Cartilage* was devised to capture important negative findings in *in vivo* models of osteoarthritis (OA), both with respect to pathogenesis and treatment of the disease. Negative results are mostly met with disappointment and dismissed by researchers as ‘failed’ experiments. It is somewhat ironic - in a disease where we collectively lack mechanistic understanding and we have no benchmarks for what is right (true) and what is wrong (false)- that we seem to deem negative studies (*i.e.*, studies where the results force us to refute our hypothesis) as being less informative than positive ones. This is concerning when we consider that the use of murine models in OA research has expanded exponentially since the turn of this century (see Figure 1), and they are increasingly being used as a screening tool for studying putative mediators of disease, often without strong mechanistic rationale.

Many of our colleagues have unpublished negative results hidden away in drawers, experiments in knockout mice that had no clear phenotype being a common example. These findings are sometimes presented in abstract format, but often never formally submitted. The responsibility of failure to publish negative findings stems in part from publishers, granting agencies and Universities who want to see positive data being delivered, and in part from the researchers themselves. Scientists may have a higher threshold for submitting negative results, since positive results are just more exciting than negative ones, and we all shy away from the demanding task of preparing a full-length manuscript that only serves to communicate “failed” experiments, owning up to the fact that we were mistaken. Even as reviewers, we may be more critical when evaluating negative studies, paying extra attention to whether the study is adequately powered and all the proper controls have been included.

The response to the call for the current Special Themed Issue on Negative *In Vivo* Studies was not overwhelming. We received 29 original research submissions, 7 of which described findings in knock-out mice, 9 submissions that tested therapies or interventions, 11 that evaluated repair strategies, and 2 others. Ultimately, just 7 original submissions were accepted, which amounts to a 25 % acceptance rate, in line with the usual acceptance rate of this Journal. We have selected a number of studies where there was a clear and compelling rationale for performing the study, where the study was robustly designed and where it tells us something important about OA pathogenesis. We have also collated four expert reviews on aspects of *in vivo* OA studies in order to improve quality, design and reporting of studies to gain optimal impact from our scientific endeavors.

Positive bias in scientific reporting is dangerous as, through citation and systematic review, it leads to an overestimate of the importance of a given pathway or treatment in disease. This contributes to failed clinical studies and the massive attrition that the pharmaceutical industry suffers during development of novel therapies. There is currently a big push toward reducing the positive bias in the scientific literature at large, also at the level of publishers, such as Elsevier <https://www.elsevier.com/authors-update/story/innovation-in-publishing/why-science-needs-to-publish-negative-results>. There are several new online journals where negative or inconclusive data can find a home (*Journal of Negative Results in Biomedicine*, *The All Results Journals*, *Journal of Articles in Support of the Null Hypothesis*, and more), and new ways to provide access to negative data have emerged (e.g., <https://pubpeer.com/>). Negative findings are also increasingly represented in broad-scope journals such as *Disease Models & Mechanisms* and *PLoS ONE* (the latter recently published a virtual collection, entitled “The Missing Pieces: A Collection of Negative, Null and Inconclusive Results”). Even so, new journals launched with the specific scope of publishing negative findings often do not attract as many papers, demonstrating that it is the underlying scientific culture that requires change and not only journal policies.

The problem is not simply in the reporting but in the quality and design of the study, as argued in the review by Smith *et al.* in this issue [1]. A false negative result is as unhelpful as a false positive result and much research funding is wasted on poorly designed studies. There are certain challenges to how we define a negative result. This cannot simply be defined by an insignificant P-value, as it will depend wholly on the size of effect the study has been powered for [2]. Our clinical colleagues can help us here: they would define a negative study as one in which the primary endpoint of the study has not been met. In other words, they decide at the outset of the study what they are going to measure, what effect size they would regard as clinically significant (and therefore relevant) and the numbers required to power the study appropriately [2]. This is rarely done in pre-clinical modeling but should be encouraged. We then have the additional problem of deciding what a clinically relevant change in cartilage degradation score is in a mouse. Most published positive studies tend to show an effect size of around 40-60%; therefore, does this mean that any effect size less than this negative?

It wouldn't be truthful to say that Rheumatology journals do not publish negative studies. There are several notable negative *in vivo* OA studies that have strongly impacted the

field. For instance, *Adamts4* null mice were found not to be protected from experimental OA [3] and *Adamts4/Adamts5* double knockout animals showed no demonstrable increased protection over *Adamts5* null mice following surgical destabilization [4]. Similarly, ablation of *Adamts1* offered no protection from accelerated aggrecan degradation in an inflammatory model of arthritis [5]. In combination with the clear protection observed in *Adamts5* null mice, both in surgical OA and in a model of inflammatory arthritis [6, 7], these findings strongly skewed efforts to block aggrecanase activity toward ADAMTS-5 as the prime target, efforts that have unfortunately not yet been translated into the clinic [8].

Published negative studies are often used to refute scientific prejudice and in the OA field this has mainly centered on the role of inflammatory molecules. Surprisingly, mice in which either IL1 β , interleukin 1 converting enzyme (ICE), MMP3, or iNOS are deleted have slightly higher disease scores rather than protection early after partial meniscectomy [9]. Lack of chondroprotection following surgical joint destabilization after deletion of the genes encoding the cyclooxygenase (COX) enzymes, COX-1 or COX-2, was reported in a Brief Report [10]. This valuable paper also carried results of two other important negative studies: lack of chondroprotection in IL1 α /IL1 β double knockout and in TNF α knockout strains. These results were reported only in the discussion with data available through the corresponding author, and thus are infrequently cited. Conversely, in a review by Glasson, modest protection in the IL1 β knockout mouse following surgical destabilization was reported [11]. Critical evaluation of these results is challenging especially as the latter paper was in a poorly accessible journal. In the current Special Issue, van Dalen and colleagues revisit IL-1 as a target in OA. Despite performing these studies in arguably a more inflammatory model of OA (collagenase induced), they show that IL-1 α / β deletion confers no reduction in cartilage loss or synovial inflammation [12]. In fact, the data suggest a trend towards increased cartilage damage in the knockout animals compared with wild type, agreeing with increased disease reported by Clements *et al.* in a surgical model [9]. On balance, despite the potent catabolic effects of IL1 on cartilage *in vitro*, IL-1 continues likely to prove a disappointing target in OA.

“What gets us into trouble is not what we don’t know, it’s what we know for sure that just ain’t so.”

– Mark Twain

We also need to be cautious about how we interpret *in vivo* models when using genetic deletion. Constitutive deletion of a gene is the most common gene-modifying tool used in mouse studies, but there are limitations associated with potential mild undetected chondrodysplasia that may impact OA severity, or compensatory mechanisms that have evolved over time. Conditional deletion of the gene of interest postnatally has the advantage of avoiding a developmental phenotype and can be temporally controlled. In this issue, two papers complemented knockout studies with overexpression of the gene of interest by adenoviral carrier [13, 14]. Both strategies failed to show a disease modifying effect following surgical joint destabilization. Timing of genetic deletion or treatment may be critical for disease outcome. In this issue, we report the failure to show a significant reduction in cartilage

degradation in *Ccl2* and *Ccr2* constitutive knockout mice [15], yet targeting the pathway after the initial injury appears to be effective at inhibiting disease [16]. In other words, molecules may have different roles at different times of disease.

There are other considerations to publishing negative data, notably ethical ones. Is it right to do an experiment and then not share knowledge from it? If an experiment was worth doing in the first place, then do we not have a duty to publish so that other groups do not end up repeating unnecessarily? Withholding data in this way goes against our desire to conform to the '3Rs' (reduction, refinement and replacement) in animal research and wastes valuable research resources, especially in our current climate where research funding is ever declining.

So what are the solutions? One simple solution would be to encourage presentation of more negative data at international meetings in abstract form; lobbying research societies to rate these as they would a good negative clinical study. We could welcome the publication of more negative data in supplementary figures of relevant papers. A more radical option would be to create an online repository (with some editorial control) where groups can deposit negative *in vivo* data with detailed experimental methodology, but without having to prepare a whole manuscript.

Ultimately, we need to change the scientific ethos in our research institutions. Imagine the following scenario:

Student: "...but what happens if my hypothesis is wrong and the study does not show a positive result?"

How should the supervisor answer?

- A. "That will be unfortunate, your chances of getting a good postdoc position will be diminished; that's the luck of the science lottery!" or
- B. "Don't worry; it's always possible to squeeze some positive result out of a negative study!" or
- C. "A negative result in a well designed and thoughtful study is always valuable!".

Conflict statement

Dr. Vincent is on the Editorial Board of OAC, and Dr. Malfait serves as an Associate Editor.

Contributions

Both authors wrote the manuscript and approved the final version.

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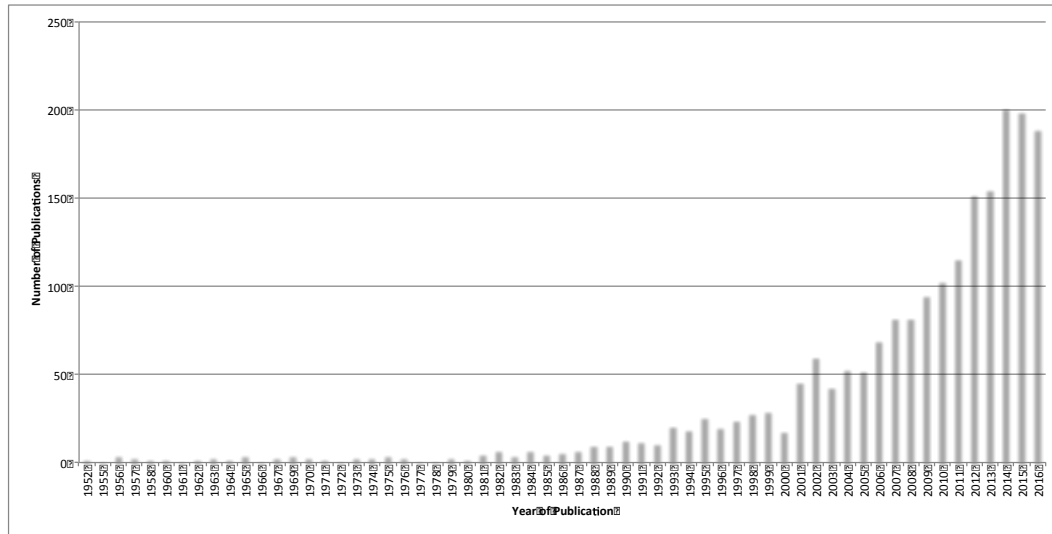


Figure 1. Number of publications by year using mice in OA research. Data was generated by searching the terms (((mouse) OR murine) OR mice) AND osteoarthritis in Pubmed, (von Loga and Vincent, “Animal models in Osteoarthritis”, in “Rheumatology” 7th Edition, in review).