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## REPLY: Evaluating Calcification in Tissue-Engineered Heart Valves

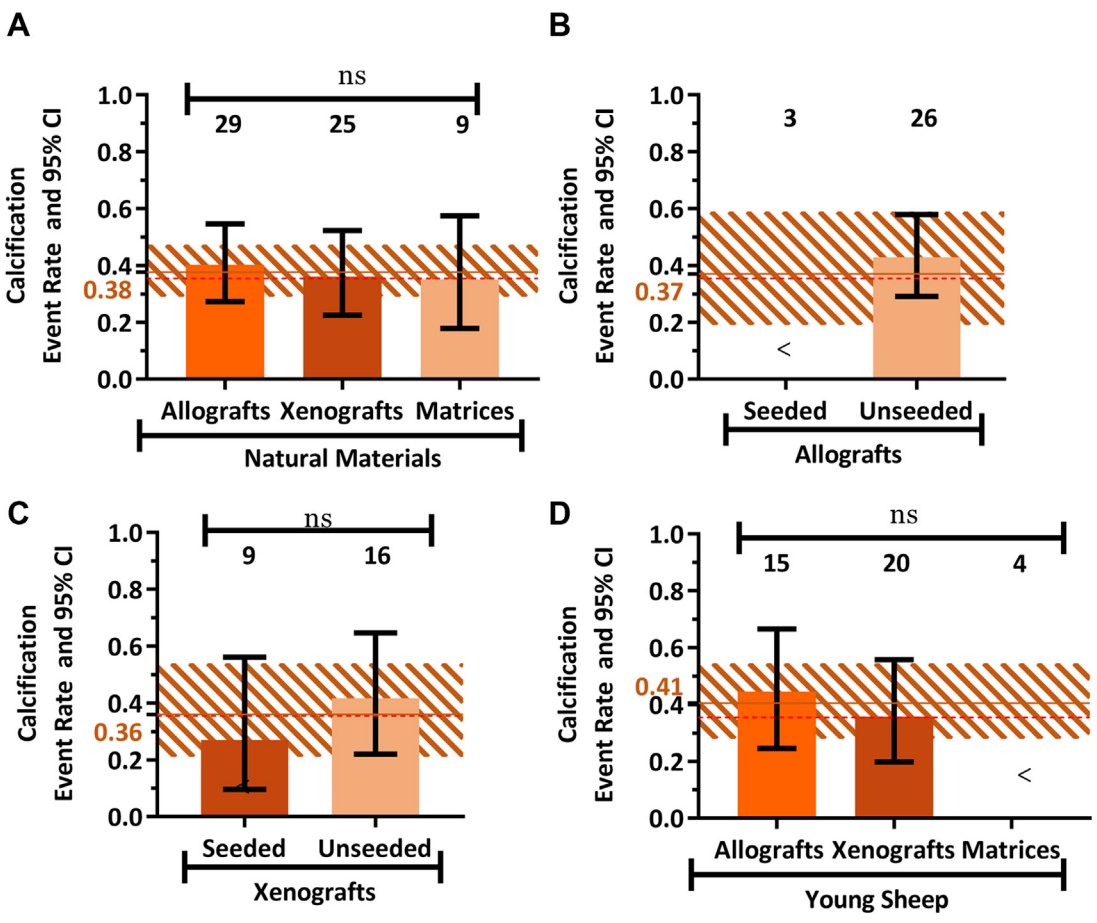
### Much More Complicated Than Expected?

Dr Motta and colleagues highlight several valuable aspects of our paper[1] that we would like to clarify and reinforce.

One important aspect raised is that our analysis cannot indicate the relevance of reported (micro) calcification in tissue-engineered heart valves (TEHVs) for pathological calcification in the clinic. Indeed—as stressed in our study—the extent of calcification reported in TEHVs is generally much milder (ie, micro to mild calcification) than clinically symptomatic calcification of native valves or bioprosthetic implants. Based on the existing data, it is impossible to predict if reported calcifications may or may not progress into clinically problematic calcification (ie, hampering valve functionality). To better understand the clinical prospects, dedicated research into the mechanisms of TEHV calcification is needed, for which our study should be conceived as a starting point.

Dr Motta and colleagues also refer to the juvenile sheep model as the most representative model of clinical valvular calcification.[2,3] Indeed, Flameng et al[2] reported that in bioprosthetic heart valves, calcification is significantly higher when studied in juvenile as compared with adult animals. However, the difference in calcification potential with age should not refrain researchers from reporting and analyzing TEHV calcification in older animals.

**FIGURE 1** Extra Sensitivity Analysis Based on Proposed Subgroups




**(A)** Subdivision of natural tissue-engineered heart valve materials into allografts, xenografts, and other natural matrices did not show any significant differences. Further subdivision of cell seeding methods in **(B)** allografts and **(C)** xenografts did not significantly change the results. **(D)** Subdivision of natural materials into allografts and xenografts within young sheep only.

Currently, limited data are available from juvenile sheep, underscoring one of the main messages of our review, namely that more dedicated and standardized preclinical research (eg, using juvenile sheep) is warranted to adequately assess calcification risk for TEHVs.

Another important point raised is the classification of TEHVs according to engineering methodology. We made a main division between methods based on the type of starter scaffold/matrix, hypothesizing this to be a key determinant of calcification risk. Our main finding is that calcification is present across all TEHV methodologies. We recognize that a further subdivision might aid better understanding of the calcification potential of these groups, but we emphasize that the aim of this study was not to compare individual TEHV techniques, but rather to give an unbiased overview of calcification in TEHVs, for which the used subdivision is most appropriate. To address the authors' suggestion, we performed an additional subgroup analysis to compare allografts and xenograft in the natural TEHV group. This did not result in different insights or differences between these groups (**Figure 1**).

As highlighted in our Discussion, the quality of our meta-analysis is dependent on the reporting quality of the original papers. Hence, an important element of our review is the assessment of study quality, specifically for analysis and reporting of calcification. Given that only 55% of studies report on calcification, our analysis is skewed in the sense that those studies that did not report on calcification were not included. It is thus important to emphasize that the inclusion of studies in our analysis does not mean that these studies show an increased risk of calcification. Rather, the included studies have a better study design and quality of reporting in terms of assessing presence or absence of calcification, which enabled inclusion in our meta-analysis.

Taken together, the main message of our systematic review is that calcification risk of TEHVs is underinvestigated and underreported. We fully agree with Motta and colleagues that standardization of study designs and reporting thereof is essential to efficiently move forward with the translation of TEHVs into a robust and safe clinically applicable treatment option.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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