ventricular mass/left ventricular mass index, ventilator-free survival or overall survival) are easy to measure and observe. Clinical outcome measures used to assess ERT response were the Medical Research Council scale and forced vital capacity. In a slowly progressive disease such as LOPD, such distinct end points take years to be evident and these measures may not be sufficiently sensitive to capture the gradual change. Moreover, these metrics may not be able to differentiate between the impact of natural disease progression versus the age-related decline in function. The Medical Research Council scale has long been criticized for its limitations, and there have been numerous attempts to improve its accuracy.

The authors speculate that the IVS1/delex18 genotype may protect against developing high antibody titers. It is known that genotype alone does not predict ERT response in Pompe disease. A number of factors, such as major histocompatibility complex class II polymorphisms, human leukocyte antigen haplotypes, the extent of non-endogenous epitopes relative to ERT, and epitope spreading (which may lead to high titers), may play a role in the treatment response. In our experience, a small fraction of CRIM-negative IPD patients do not develop high antibody titers and respond favorably to ERT, which suggests that genotype alone is not responsible for the immune response.[4] Thus, the observation of genotype association in LOPD should be stated with caution.

In summary, we believe that the persistence of elevated titers over time, rather than the absolute values at a single time point, is a key predictor of clinical outcomes. It remains to be examined whether a complete elimination of antibody formation from the time of ERT initiation would change the outcome. Outcome measures that have the ability to capture small changes in LOPD need to be developed.

DISCLOSURE

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Response to Herbert *et al.*

To the Editor: We thank Herbert et al.[1] for their interest in our work.[2] Their laboratory has shown to be instrumental in studying the effects of enzyme replacement therapy (ERT) in infants with Pompe disease. However, there are some misunderstandings about our study on adult Pompe patients and antibody formation. Below we explain these in detail.

Herbert et al.[1] suggest that our patients be reclassified based on sustained titers rather than peak titers, because "the current classification based on a single, maximal value may lead to a lack of clarity on the role of antibody titers". We agree that the duration of high neutralizing antibody titers is important to consider. This is why we measured titers at multiple time points over a period of 3 years (Figures 1 and 3, and Supplementary Figure 1A–C in de Vries et al.[2]). We observed two trends: (i) a decline of peak titers over this period at a group level and (ii) relatively few patients with high (≥31,250) peak antibody titers (16 of 73; 22%). Nine (12%) of these had high sustained antibody titers. Eight patients (11%) had very high (≥156,250) peak titers, and these classified for all but one patient as sustained high. This shows that no matter how the groups are generated, in all of these cases group sizes are very small. The statistical power to analyze potential effects on clinical outcome is limited. Therefore, we have also analyzed the eight patients with a very high peak titer and seven patients with a high sustained titer on an individual basis, and we concluded that antibodies were likely to have interfered with the effect of ERT in only one patient. We previously reported on the counteracting effect of high sustained antibodies in this particular patient.[3] Herbert and colleagues may have missed the fact that only a few adult patients develop high sustained antibodies, which is in contrast to the situation in classic infantile patients. A recent study by Masat et al.[4] on behalf of the French Pompe Registry Study Group also concluded that antibodies are not a major concern in adults with Pompe disease.[4]

Herbert et al.[1] note that not all cross-reactive immunologic material–negative infantile Pompe patients developed neutralizing antibodies, while they all experienced poor clinical outcome, and that the role of neutralizing antibodies should not be overlooked. We agree that neutralizing antibodies are not the only explanation for a poor response to ERT. First, ERT does not compensate in all cases for α-glucosidase (GAA) deficiency to an activity level above the critical threshold. The reason for this could be that the dosage is too low or the therapy is inefficient due to the formation of anti-recombinant human GAA antibodies, which neutralize GAA activity and/or interfere with cellular uptake. Second, Pompe disease may have progressed too far and tissue damage has become beyond repair. Third, as yet unknown modifying factors may enhance or decrease the effect of ERT. Fourth, the lysosomal storage of glycogen in Pompe disease induces secondary cellular responses, such as a block of autophagic flux and mitochondrial dysfunction—processes bound to interfere with ERT. Evidently, antibodies are just one of several factors determining the outcome of ERT. This is also emphasized by the heterogeneous response to ERT in patients with no or low antibody titers in our study.

Herbert and colleagues[1] suggest that assay variability "appears to be associated with control reagents" rather than titering patient samples. It is unclear to us why the authors conclude this as this is misconstrued from our paper; we did use patient samples over the titer range to determine assay variability.

Herbert et al.[1] question whether the assay used in our study to measure neutralizing effects has been standardized and whether the cellular matrix could cause variability. The assay has been standardized and the same cellular matrix (fibroblasts from a classic infantile patient without any detectable GAA activity) was used in all experiments. We would like to emphasize that assessment of neutralizing effects is an important aspect to investigate the potential impact of antibodies on ERT, and we wish to promote its assessment as a standard assay whenever high antibody titers are found.

The authors also question the use of our clinical outcome measures as a readout for efficacy. We note that the outcome measures have been internationally recognized in consensus meetings and have been found suitable for the detection of changes in patient performance in response to ERT in multiple clinical studies. We recommend testing for the presence of neutralizing antibodies in the case of infusion-associated reactions and when clinical outcome declines.

Herbert et al.[1] state that "genotype alone is not responsible for immune response" and that "the observation of genotype association in LOPD should be stated with caution". We regret what appears to be a misunderstanding of our work. We did not state in our article that genotype alone is responsible for the immune response. We did, however, state that our results should be confirmed in a larger patient group.

In summary, we have conducted an in-depth study in which we measured antibody titers and their neutralizing effects at multiple time points over a period of 3 years. This showed that titers declined on a group level, a limited number of patients developed high antibody titers, and a subset of these patients showed high sustained titers, but in only one patient was a clear impact of antibodies on the effect of ERT likely.

DISCLOSURE

A.T.vdP. has provided consulting services for various industries in the field of Pompe disease under an agreement between these industries and Erasmus MC. The other authors declare no conflict of interest.

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Pitfalls of trio-based exome sequencing: imprinted genes and parental mosaicism—*MAGEL2* as an example

To the Editor: Family-based whole-exome sequencing has proven to be an effective diagnostic strategy for the identification of causative variants in individuals with intellectual disability (ID) and congenital malformations