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Chances and obstacles of pilot projects in animal research for statistical sample size calculation

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ABSTRACT

Animal experiments are a core element of basic medical research. Their accurate planning regarding sample size, however, poses a challenge from a biometrical point of view, since valid information is rarely given. One proposed concept includes the integration of pilot projects in animal research to encounter this shortcoming. The undoubtedly resulting advantages of this approach regarding the estimation of the required sample size though have to be discussed critically and should be contrasted to potential obstacles and practical implications, respectively.

1. Introduction

Animal experiments are hardly replaceable in basic medical research. Their results initiate clinical phase I studies or the whole process of clinical research, respectively, and often the respecting investigations could not appropriately be performed in an in vitro experiment. Therefore, high quality results should be aimed in the course of an animal experiment. But there are not only quality aspects which require a faithful examination of the contentual and methodological issues in planning these projects, especially ethical aspects has to be considered at all projects [11]. The ethical tenability of animal experiments in basic medical research is discussed controversial in public [7,16]. Therefore, people in authority of the projects have to seize and use adequate measures to minimize the suffering of experimental animals on the one hand and to optimize the total number of animals on the other hand. This optimization means to find a total number of experimental animals which is as few as possible (to be able to show a desired effect) and which is as much as is necessary otherwise (to be able to detect a desired effect). From a biometrical point of view, the statistical sample size calculation and the required information for it play a central role in these considerations of optimization.

In accordance with the responsible animal welfare commission for the University of Ulm, "in basic medical research there must be no other demands with respect to biometrical planning compared to applied research in humans" (i.e. the study phases I-III). A core element of that planning is a previous statistical estimation of the required sample size. This estimation, however, is in animal experiments not automatically possible, since the requisite information for it is missing or its transferability from other project is doubtful. One proposed concept of the animal welfare commission to handle this problem includes a distinction between "pilot studies (so called exploration experiments) and exact large-scale experiments".

According to this, pilot studies should be requested if there is no information with respect to the investigated research question and therefore no statistical estimation of sample size is possible. Large-scale experiments are classified as projects for which the requested sample size can be verified computationally based on available information. It is conceivable that this information arises from comparable projects or from previously requested pilot projects. In principle, the concept of pilot studies in animal research promises several crucial advantages [17] from a biometrical view, but these have to be contrasted to certain constraints.

In this article, especially the advantages and drawbacks, with respect to the statistical sample size estimation, are discussed which arise from integrated pilot projects within the process of animal research. The biometrical aspects of sample size calculation are focussed, particularly the application of appropriate methods and the possibility of using adaptive study designs with integrated pilot study.

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Chances owing to integrated pilot studies in animal research

By differentiating pilot projects from large-scale experiments, which are predominantly requested in Germany, the animal welfare commission aims especially an optimization of the animal research process which relates not exclusively to an optimization of utilized animals. Moreover, the process of application and planning of the experiments in animals should become more understandable.

Structural aspects

In Germany, all relevant project information has to be provided by the applicant for the appraisal of an animal experiment. With respect to clarity, structure and intelligibility those applications differ from each other tremendously in parts. There are indeed respective templates, but planning the experiments is the applicants' task themselves. It is prescribed for the applicants to contact the animal welfare commissioner of the associated research institution, best at the very beginning of the project, to identify and avoid potential weak spots of the experimental design. Further constructive advices also arise from a statistical consulting and appraisal. Above all, a clear structure of the applications is sought by these consultations, because a comprehensible structure simplifies the work of all involved parties within the appraisal process, especially for the animal welfare commission which examines the application form as the last link in the chain to give its approval or refusal.

In practice, however, the experiments often include multiple sub-experiments and are therefore very complex. As a result, intelligibility of the experimental design is reduced. The first sub-experiments are often used to fix the ultimately necessary sample size for the so called "main experiment". This proceeding results not only in a uncertain sample size determination, but additionally in a more complex structure of the whole experiment. An outsourcing of those sub-experiments as distinct pilot projects would simplify both aspects.

Reliable base for large-scale experiments

Projects of basic medical research have the general problem of hardly existent information which can promptly be used to plan an experiment. On the one hand, experiment replicates are limited to specific situations [8] according to animal welfare, on the other hand the transferability of information from similar experiments has to be carefully checked if it should serve as a foundation of planning. Here, the statistical planning of required sample size is central.

The implementation of pilot studies in the process of basic medical research ensures an appropriate transferability of previous information without going back to experiment replicates as such. According to the responsible animal welfare commission for the University of Ulm, pilot experiments are especially different from large-scale experiments with respect to the maximal approvable number of animals. This number is limited in pilot projects to "80 to 100 animals", whereas the number of animals for (subsequent) large-scale experiments is unlimited. The pilot experiment is used to prove if there is generally a chance to reach the aimed goal within the large-scale experiment. Thus, by establishing pilot experiments, a proceeding analogous to clinical trials is possible in animal research as well.

The results of a pilot experiment can be used as a valid planning basis, especially with respect to the estimation of an optimal sample size for a subsequent large-scale experiment.

Purposeful statistical sample size estimation

The statistical verification of requested sample size can be thought of as a core element of biometrical assessments. Although their submission at the approval agencies is not mandatory according to current guidelines [1,8], the submitted applications are usually processed only if a biometrical statement is provided additionally. The necessity of these biometrical assessments is not regulated within legal guidelines, so their form and content is free. However, the biometrical statement should enable to understand how the requested number of animals comes about and that this sample size is optimal for the respective project. This information should also be provided within a related publication of the in vivo results according to the ARRIVE guideline [14] which has been published in 2010.

The method of choice at that is the statistical sample size estimation from a biometrical view [11]. Based on respective previous information, the least necessary and simultaneously most required sample size is calculated. For the validity of such estimations, a high transferability of the data is crucial [5]. Animal experiments are a part of basic medical research and are therefore at the beginning of the research process, however, so valid information is hardly available. If the data for planning arise from similar experiments, there is maybe a problem of transferability. Moreover, often in such a situation the information is not appropriate with respect to the adequate methodology of sample size calculation, e.g. if data are only available for a two-group comparison, although the analysis strategy requires a multifactorial setting due to the conception of the experiment.

By establishing a pilot project, the planning of a subsequent large-scale experiment can be based on data which are valid and transferable. Moreover, the methodologies for planning and analysis coincide.

Advantages of a detailed planning base

To illustrate the problem of disaccording methods of planning and analysis, which often has to be traced back to unavailable detailed information, the following example is presented. It describes a common situation where the experimental design requires a multifactorial analysis strategy.

An animal experiment examines the impact of IL-20 homologous cytokines to the immune system. Influential factors are the two-stage variables genotype, gender and time point (i.e. a three-factorial setting), whereas the outcome variable is continuous. An adequate analysis strategy for this experiment would be a ANOVA under the assumption of normally distributed data with homogenous variances or non-parametric approaches according to Brunner and Puri [6], respectively, as an alternative free of distributional assumptions.

To demonstrate that the accordance of planning and analysis methodology has a relevant impact on the calculation of sample sizes, two different scenarios are assumed:

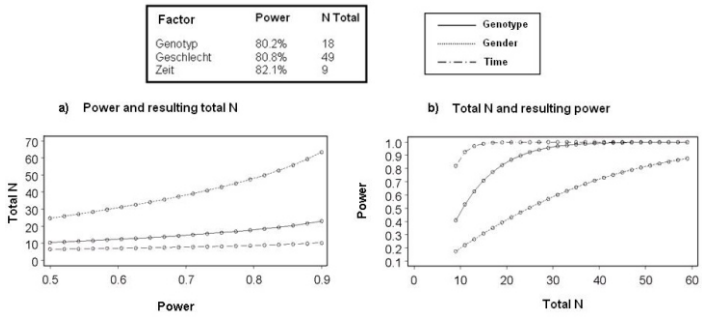
(1) All available information with respect to the three factors are considered for sample size calculation in an ANOVA setting (without interaction terms and without adjustment of the significance level). The procedure PROC GLMPOWER in the statistical software SAS is used.

(2) It is assumed that only information about the genotype difference for a specific time point is available. Hence, the planning is based on a two-group comparison (t-test, given that the data are normally distributed). The procedure PROC POWER of the SAS software is used for that.

Both scenarios illustrate two different situations of sample size calculation for animal experiments: (2) conforms to the common situation in practice with just vestigial information. Scenario (1), however, represents a situation as it would be possible to realize by establishing pilot projects.

Assuming normally distributed data, a statistical power of 80% and a two-sided significance level of 5%, results of the sample size calculations for the two scenarios are presented in the following.

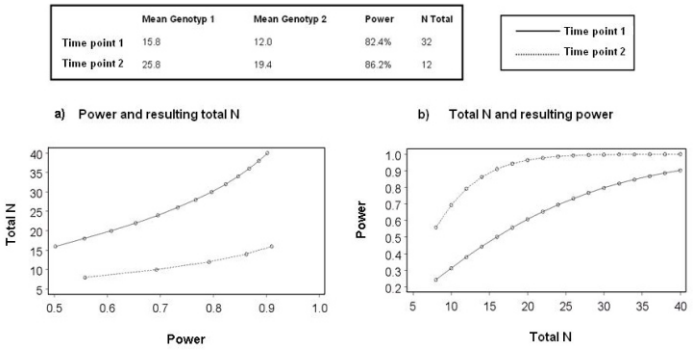
Figure 1: Sample size calculation with a three-factorial ANOVA design



Obviously, the three factors have a different impact on the necessary sample size. To find differences in the outcome variable with respect to the factor time, 9 animals (i.e. 5 per group) would be sufficient. In contrast, 49 animals (25 per group) would be necessary to show gender-specific differences in the outcome variable. The most important comparison with respect to the investigated genotypes would result in 18 animals in total (9 per group). These findings can also be seen in figure 1, while in scenario (1) a standard deviation of σ=3.6 units of the outcome variable is assumed.

In (2) the same data are used as in scenario (1), but here the assumption is made that sample size estimation is only possible to find genotype-specific differences for a certain time point. This represents a common situation in practice. The two distinct time points of (1) are considered separately for sample size estimation, where the calculation of the means of the outcome variable ist based on both genders and the same standard deviation of σ=3.6 is assumed.

Figure 2: Sample size calculation for a difference in genotypes with a two-group comparison (t-Test)



The calculated sample sizes differ noticeably from those in scenario (1). In particular, the sample size to detect genotype-specific differences has been 18 animals in (1) and is now 12 (at a later time point) or 32 (at an earlier time point) animals, respectively. In case of a later time point the planning would suggest less animals than in (1) which in consequence would lead to non significant results in an ANOVA analysis with respect to genotype-specific differences in the outcome variable. In case of an earlier time point, however, more animals than in (1) are proposed. Therefore, more animals than necessary would be used in an ANOVA analysis to find significant differences between genotypes.

In total, this data example illustrates that the usage of different planning methodologies may lead to substantial differences in estimated sample size, so the importance of an adequate methodology is emphasized. The establishment of pilot projects enables such an adequate planning.

Available biometrical methods

In medical statistics there are respective methods to deal with integrated pilot studies, especially with respect to sample size calculation. These are so called adaptive study designs which can be handled with the software ADDPLAN, for example. This program has been developed for planning and analysis of adaptive and group sequential study designs.

3. Obstacles for integrated projects in animal experiments

The just mentioned advantages of integrated pilot experiments in animal research of course have to be contrasted to some possible negative aspects of this approach. An implementation of pilot projects forces researcher to rethink their scientific proceeding which perhaps is not exactly a simplification of the research

process. Apart from that, integrated pilot experiments bring along further debatable aspects and implications with respect to the statistical methodology for an adequate planning of sample size.

Researcher's determining factors

Animal experiments are a core element of many research groups in basic medical research. So, their employees depend on respective projects which are approved to answer the intended research questions. The strategy of requesting large projects with high amounts of animals and sub projects, respectively, enables research groups to have a temporal structure that is quite steady and reliable to ensure the subsequent academic activity after approval. This is a very important aspect with respect to intermediate-term equipment of research groups with scientific staff (researchers, doctoral students, student assistants). An intermediate-term planning security is not only important for the institutions themselves, however, but rather for the logistic organization of the animal experiments, e.g. with respect to keeping of animals. If there is a central animal husbandry, the projects have to be also arranged with the capacities of experimental animal husbandry, so the logistic organization as well as the internal organization of research groups benefit from a intermediate-term planning security. The implementation of pilot projects, however, is therefore adverse for these organizational issues, since experiments with more animals or long-ranging projects depend on the results of a previously conducted pilot experiment. Ultimately, these circumstances may lead to more difficulties in acquiring external funds which are often used to sponsor the projects partially, because the chances of success are highly dependent on the results of a respective pilot experiment.

Methodological aspects

From a biometrical point of view, the implementation of pilot projects in animal research has to be discussed with respect to sample size calculation, especially for entire projects including pilot and subsequent large-scale experiments. As already posed, adequate pilot experiments can be used for the generation of valid information which serves as the base for large-scale projects. From a biometrical view this separation of entire projects implies the application of an adaptive study design [3,10]. These divide experiments in a learning and a confirmation phase [2] and use the information from the first phase to adapt the subsequent experiment, e.g. with respect to necessary sample size. Beside the advantages and drawbacks which are described in [2,4], an adaptive design in animal research would mean that the necessary sample size for the entire projects remains unclear until a respective interim analysis. In terms of to be able to plan an animal experiment this circumstance means a dramatic limitation for affected research groups because of the just mentioned determining factors.

Additionally, one has to think of how the animals of a pilot experiment should be handled when applying adaptive designs. For the purpose of an adaptive, group sequential design of the entire project, composed of a pilot experiment and a subsequent large-scale experiment, the animals of the pilot project are a part of the total amount of animals for the entire project. Therefore, these animals have to be considered as applicable sample size within the large-scale project. The sample size calculation for the large-scale trial then has to consider the results of the previous pilot experiment in accordance with a correction of the significance level due to multiple testing [12,13,15]. Creating the same experimental conditions, especially regarding the applied animals, is a very difficult issue in animal experiments, however, since often animals of the same kindle have to be used. Due to the chronological distance between the two experimental phases, this cannot guaranteed in general. Overall, a methodologically correct consideration of already surveyed values of a pilot project requires an explicit usage of adaptive designs, which can hardly be implemented in animal experiments.

Limitation of maximal sample size

From applicants point of view, the limitation of the number of animals for a pilot experiment constitutes a major problem. An animal experiment tries to investigate several potentially relevant research questions to figure out the most interesting which subsequently are pursued in further projects (e.g. phase I studies) [9]. Statistically this implies multifactorial experimental designs in which the number of investigational groups can rise very fast. Keeping in mind that in each group at least five animals should be included to be able to get reasonable results, a number of e.g. 100 animals is reached quickly. Such an amount of animals can serve as such an upper limit.

Confinement of the explorative character of research

In one sense, the limitation of maximal sample size for pilot studies constitutes a limitation of the experimental character of basic medical research. Often a discovered effect estimate of a previously less observed “derivative” of the primary research question proves interesting for subsequent investigations. But if one has to focus very early particular questions to comply with the maximal sample size for pilot experiments, maybe some potentially important results are lost or remain undetected, respectively. Ultimately, the explorative conception of basic medical research is an indispensable producer of novel insights and is therefore the engine of innovative research concepts.

4. Discussion

In general, the implementation of integrated pilot experiments in animal research would have some substantial advantages with respect to a funded planning of the projects. According to the opinion of the animal welfare commission for the University of Ulm, in basic medical research there have to be the same standards like in clinical research to reach highly validated results. Especially in animal research, ethically and methodologically effective determining factors have to be considered. As less as possible animals should be used to obtain meaningful results. Since animal experiments basically cannot be based on much prior information for planning the projects, the implementation of pilot projects is a useful approach to solve this problem. The crucial advantages from a structural and statistical point of view have been discussed.

However, adverse issues of such a proceeding have to be considered, too. From methodological view, adaptive designs should be used for sample size calculations with respect to the entire project. Here, numerous obstacles regarding planning security and limitation of the explorative character of animal experiments have been discussed. Most fatal is the limitation of the maximal approvable sample size for pilot experiments which restricts the scope of research groups. But this must not be misunderstood insofar that in animal experiments there should be a scope for decision-making that lies beyond all measures with respect to the amount of animals. In accordance to animal welfare, applicants have to prove by biometrical assessments, for example, that the requested sample size is optimal for the planned project. Anyhow, despite the implementation of integrated pilot studies in the process of basic medical research it should be attempted to keep the explorative character of this field of medical research to the greatest possible extent.

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