The Role of Population Size in Computational Assessment of Pharmacological Cardiotoxicity

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Abstract

In the context of computational electrophysiological simulations aimed at assessing pharmacological cardiotoxicity, several parameters play crucial roles. Among theme, the size of the population under scrutiny emerges as potentially critical, negatively impacting on the expected final outcomes.

Particularly noteworthy is the lack of a definitive standards specifying the optimal sample size to be employed. This study attempts to shed light on the influence exerted by population size on the arrhythmic risk classification of drugs.

Initially, a population of 100,000 stable virtual cardiomyocytes was generated. From this initial population, ten different sets of 2989 stable models were extracted, which were subjected to cardiotoxicity analysis. Subsequently, a bootstrapping process was performed, involving the random extraction of 107 cells from a one of the sets of 2989 models. The operation was repeated 100 times for statistical significance.

The study reveals a greater variability within the population of 107 cells as opposed to the larger cohort of 2989 cells. This discrepancy is to highlight the potential for considerable errors in pharmacological cardiotoxicity classification deriving from the utilization of inadequate sample sizes.

1. Introduction

When conducting studies on pharmacological cardiotoxicity, numerous parameters must be considered to ensure a reliable and accurate analysis. Among these parameters, the characteristics of the population are of particular interest. It is crucial not only to know the age, gender, health status, and comorbidities of the population but also to carefully consider the population size itself. Choosing a not sufficiently large population could lead to misleading interpretation of the results and data, and misleading conclusions.

In the field of electrophysiology, particularly concerning the evaluation of drug-induced cardiotoxicity, the risk of errors related to population size selection is further exacerbated by the lack of a standard methodology. Despite considerable efforts since 2010 [1] to move toward an in-silico paradigm to assess pharmacological cardiotoxicity, there are still no established standards for good in silico practice.

Moreover, there are no universally accepted guidelines for the correct characterization of parameters in in-silico pharmacological cardiotoxicity assessments, where population size plays a critical role. In the context of pharmacological cardiotoxicity, the term "population" typically refers to the cellular population, i.e., the number of cells used to conduct a given in silico study. In literature there are a number of in silico cardiotoxicity assessment utilizing different sample sizes. For instance, in [2] a pathological population of 107 cells is used, where as other studies propose larger population [3,4,5,6]. Moreover, [2,3] showed that small population size, by optimizing the space parameters, can yield similar outcomes i.e., the risk index associated with a drug. To conduct an adequate and representative analysis of human cardiac electrophysiology, it becomes mandatory to construct a population that is as heterogeneous as possible [9,10] while remaining within the pathophysiological context of interest and statistically significant. It is now evident that as the number of cells increases, so does the computational time required to conduct the analysis. Therefore, considering the relationship between population size and computational cost, it becomes immediately apparent how important it is to establish a standard that identifies the optimal sample size and, more importantly, to determine whether using large or small populations affect minimally the final assessment of pharmacological cardiotoxicity. As we will discuss in the results and discussion sections, it is not so straightforward to assert that small population size has the same or similar effect of large ones on the risk index of a given drug.

2. Methods

This study aims at investigating the effect of population size in the context of a pharmacological cardiotoxicity analysis using the isolated cell model.

2.1. Calibration of the Population and Risk Analysis Framework

From the initial 100,000 ventricular cardiomyocytes, a subset of 2989 cells were selected by calibrating the population against experimental data from [11], focusing on key action potential biomarkers, such as Action Potential Duration at 90%, 50%, and 20% repolarization (APD90, APD50, APD20), resting membrane potential (RMP), and action potential amplitude (APA). Subsequently, the cardiotoxicity characteristics of three drugs: *Amiodarone*, *Diltiazem*, and *Quinidine* were investigated. Each drug belongs to a different risk class: Borderline, Non-proarrhythmic, and Proarrhythmic, respectively [2] .

For the Arrhythmic Risk Score (ARS) analysis and subsequent classification, the following formula was employed to characterize the number of pro-arrhythmic events relative to the total number of events:

$$ARS = \frac{\sum_c (W_c \cdot nAE_c)}{N \cdot \sum_c W_c} \quad (1)$$

Where $\sum_c W_c$ is the sum on all concentration, [C] is the concentration under consideration, W_c=EFTPC/[C], N is the total number of models in the population, and nAE_c is the number of models showing pro-arrhythmic events determined by the detection of specific abnormalities following drug administration, including spontaneous excitability (dV/dt > 0 during the resting phase), an increase in APD90 exceeding 20% relative to baseline control potentials, non-physiological resting membrane potential values, abnormal peak values, cardiac alternans, early afterdepolarizations (EADs), and unusual triangulation values, calculated as (APD90 - APD30) [12].

2.2 Statistical Analysis

To address the question posed in the introduction regarding the importance of population size, a bootstrapping process was performed on the 2989 cells, randomly selecting 107 cells. To ensure the robustness of the selection, a sensitivity analysis was conducted on the number of iterations necessary to guarantee statistical significance. Therefore, the extraction was repeated 1, 10, 50, 100, 200, 300, and 600 times, and for each extraction class, the statistical distribution of the respective risk indices of the 107 cells was calculated, along with the associated median and interquartile range. After identifying the threshold value for the number of iterations required to achieve statistical significance, two additional aspects related to population size were investigated. The first aspect involves comparing the ARS (Arrhythmic Risk Score) of the population of 2989 cells with the extracted subpopulation of 107 cells, followed by an analysis to determine the threshold sample size of the subpopulation to avoid underestimating the actual behavior of the population. The second aspect, which is much more computationally demanding, involves repeating the entire process of generating the 2989 cells from the initial population of 100,000 without extracting subpopulations. This process encompasses the entire evaluation of cardiotoxicity, including all related computational simulations. In this study, the statistical behavior was examined with 10 iterations for the three aforementioned drugs, and then the number of iterations was increased to 50 to verify that variability did not increase.

3. Results

For all three drugs, a sensitivity analysis was performed to determine the number of iterations necessary to achieve statistical significance. As anticipated, the analysis revealed that 1 and 10 iterations are insufficient, leading to highly variable results.

However, from 50 iterations on, a convergence of the results was observed, indicating that this number of iterations is adequate for statistical significance.

Regarding the risk index comparison between 107 cells and 2989 cells, the results demonstrate that reducing the number of cells from 2989 to a subpopulation of 107 cells increases variability.

Specifically, for *Quinidine*, a drug previously classified as pro-arrhythmic [2,4], the variability remains almost entirely unchanged between the two cases, reason why here was not reported in figure 2.

Conversely, for *Amiodarone* and *Diltiazem*, the range of variability becomes considerably larger with variances reported in Table 1 for both 2989 cells and 107 cells. The trends highlighted in the table are reported in Figure 2, where it is evident that the variability increases when using a smaller population compared to the original 2989 cells, particularly for *Amiodarone* and *Diltiazem*. However, for *Quinidine*, the variability is almost negligible, with nearly a 100% probability of generating pro-arrhythmic events. Additionally, the study examined how variability behaves when gradually extracting a smaller number of cells until reaching the largest significant extraction from the population of 2989 cells. As expected, the results show a gradual

Table 1. The table reports the median and variances for each drug at 107 and 2989 cells

Drugs	Median 107 cells	$S^2_{107\ cells}$	Median 2989 cells	$\sigma^2_{2989\ cells}$
Amiodarone	0.139	$5.65 \cdot 10^{-4}$	0.132	$1.53 \cdot 10^{-5}$
Diltiazem	0.170	$5.93 \cdot 10^{-4}$	0.169	$1.37 \cdot 10^{-5}$
Quinidine	1	$6.16 \cdot 10^{-6}$	0.9995	$2.17 \cdot 10^{-7}$

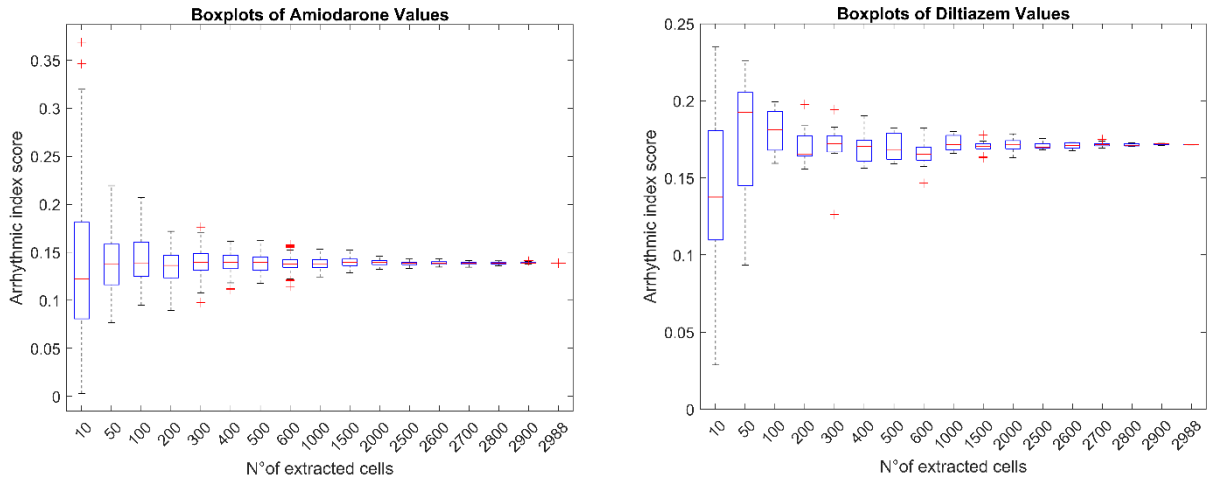

Figure 1. Here are reported, for the three drugs, the variation of the ARS as a function of the number of extracted cells with corresponding statistical variability.

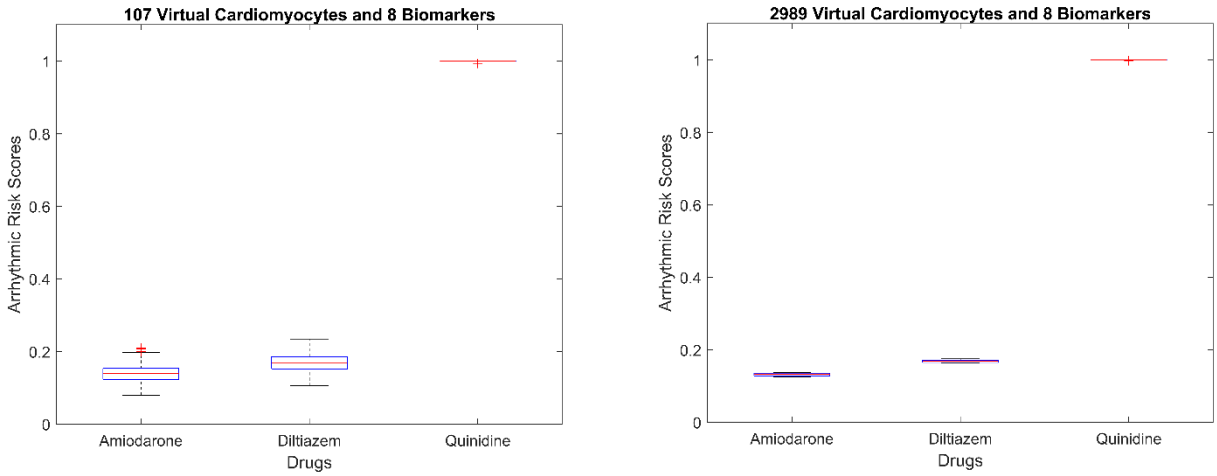

Figure 1. In figure are depicted the box plots of Arrhythmic Risk Scores of *Amiodarone*, *Diltiazem* and *Quinidine* related to 2989 and 107 cells

decrease in variability, starting from relatively high values and eventually reaching the intrinsic variability seen in the 10 and 50 simulation iterations with 2989 cells. As seen in the graphs, with the exception of Quinidine where variability remains nearly constant across all sampling methods, the variability for Amiodarone and Diltiazem increases drastically as the population size decreases. While there is no clear threshold below which variability becomes excessively high, it is evident that when the sample size falls below 200 cells, the sample size plays a predominant role in significantly increasing the variability of the results.

This can potentially increase the probability of inducing a pro-arrhythmic event by as much as 10%.

For non-normal distributions, to confirm the statistical significance allowing to comparing the variances the Brown-Forsythe test has been performed ($p<0.05$).

4. Discussion

Firstly, this study analyzed only three drugs, primarily due to computational cost constraints. Given that such a statistical analysis inevitably involves a large number of simulations, which must be repeated not only based on the number of drugs but also for each varied parameter such as the number of iterations, the number of extracted cells, and, most importantly, the number of times the population of 2989 cells is generated focusing on three drugs, each representing a different risk class, was a reasonable trade-off between cost and benefit.

Secondly, the study highlights the significant efforts required to standardize one of the critical parameters in pharmacological cardiotoxicity studies: population size.

It is evident that it is not possible to definitively establish a single sample size that is sufficient to conduct such studies with both accuracy and reliability.

However, it is essential to emphasize the need to test a sample size of no fewer than 200 cells; otherwise, there is a high likelihood of obtaining highly variable results in terms of ARS. This observation is particularly applicable to drugs that are not widely recognized as pro-arrhythmic.

It seems, in fact, to be easier to characterize the pro-arrhythmic potential of a drug than its non-pro-arrhythmic potential, with the associated variability being higher for drugs that do not belong to the pro-arrhythmic risk class. The variability associated with sample size, as well as other parameters such as the number and type of biomarkers, the electrophysiological model adopted, and the type of population, is so significant that it can lead to vastly different conclusions. Moreover, the combined effect of these factors could generate such high variability that it might result in a completely incorrect classification of the drug's risk profile.

5. Conclusion and Future developments

In conclusion, this study has highlighted how the intrinsic variability associated with choosing the population size can significantly impact the classification of cardiotoxic risk related to pharmacological compounds. Specifically, using a population size that is too small leads to a high degree of variability in the results, potentially causing misinterpretations in the final outcomes.

This work represents only a preliminary study and should be expanded to include a larger number of drugs, as well as an increased number of iterations for generating the control population. This would allow for a more thorough examination of how the control population statistically deviates from the initial general population of 100,000 ventricular cardiomyocytes.

Future research in this area is essential to develop more robust and standardized approaches for in silico pharmacological cardiotoxicity assessments.

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