Optimal cycle thresholds for COVID-19 screening – ROC-based methods highlight between-study differences

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Dear Editor,

Several articles in recent editions of this journal have addressed questions regarding the role of cycle thresholds in the interpretation of molecular PCR-based tests for COVID-19 [1–3]. The reason for this is that there is now a robust evidence-base for the assertion that cycle thresholds are related to infectiousness, the development of an immune response, and symptom severity. However, translating this knowledge about significant relationships between cycle-thresholds and relevant clinical outcomes into a specific threshold, e.g. 27, 30, or 35, requires different analytic methods than the ones that have hitherto been used in the empirical literature. Specifically, we propose using receiver-operating-characteristic (ROC)-based methods [4,5] to first determine empirically justified optimal cut off scores for cycle thresholds and second, to test their generality by comparing different studies.

In order to demonstrate the utility of this approach, we contacted the corresponding authors of the studies identified by Jefferson and colleagues [6] as reporting on the association between cycle thresholds and viral culture positivity. Of the eight authors contacted two responded and provided the necessary data [5,6]. For two additional studies [7,8] we were able to extract this data from the figures which showed the cycle threshold and viral culture positivity in the published articles. We then analyzed this data using the *cutpointr* [5] package for the open-source software R. Specifically, we plotted the distribution of cycle thresholds in culture positive and culture negative patients across studies, the ROC-curve for the four studies, the cut points identified as optimal (criterion minimum 95% detection of virus-positive culture) and the out-of-bag estimation for the AUC.

As can be seen in figure 1 there are marked differences between the studies. Most importantly the cycle threshold scores that are identified as optimal range from 26 [95%-CI: 22-32] [8] to 37 [95%-CI:34- 39] [7], while the other two studies provide optimal cut points of 29 [95%-CI:26-29] [3] and 31 [95%-CI:31-31] [2]. The confidence intervals indicate that estimation of optimal cut points is prone to random errors and that the differences between the studies are larger than can simply be attributed to chance.

While our analysis is limited by the poor data availability our results already provide evidence for systematic differences in the optimal cycle thresholds. Therefore, great care is required when deciding which threshold should be used to determine whether a person is COVID-19 positive or negative. In addition, the width of the confidence intervals demonstrates that estimates of optimal cut points need to be based on very large samples. We believe that ROC-based methods are a valuable addition to the methodological toolkit because they allow formulating explicit criteria for what constitutes optimal cycle thresholds. Furthermore, while others have speculated before that it might not be possible to determine an universally applicable threshold [9], the methods sketched above [5] allow a more precise answer to the question which PCR-tests may use similar cycle thresholds.

Potential conflicts of interest

The authors declare no conflicts of interest.

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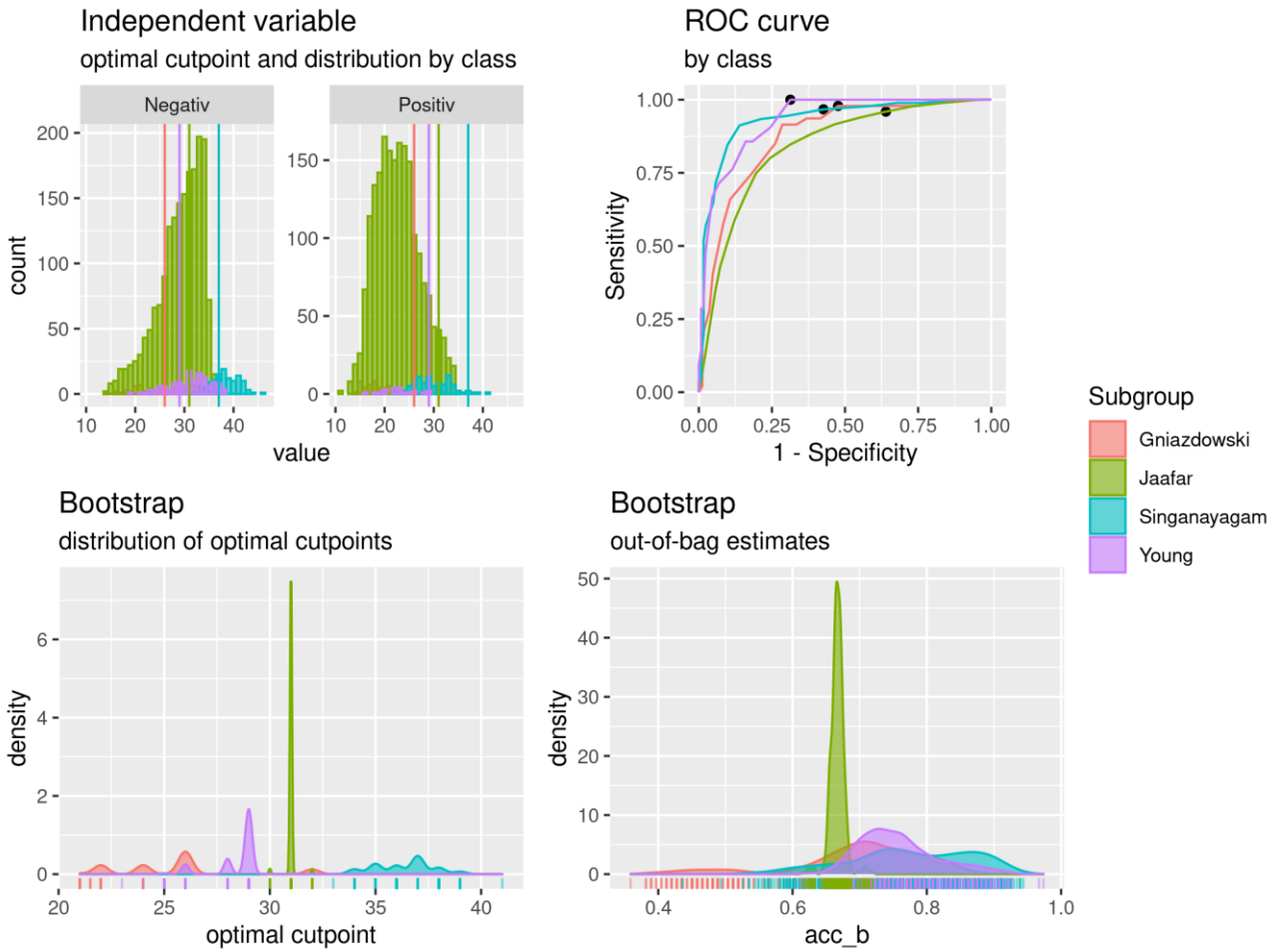
Figure Legends

Figure 1. Optimal cut off scores for cycle thresholds.

Figure 1


Independent variable
optimal cutpoint and distribution by class
Negativ
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ROC curve
by class
Sensitivity
1 - Specificity
Subgroup
Gniazdowski
Jaafar
Singanayagam
Young
Bootstrap
distribution of optimal cutpoints
density
optimal cutpoint
Bootstrap
out-of-bag estimates
density
acc_b