Comparing antigenic distance metrics for influenza

EPID 8200 project

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Abstract

Developing universal influenza vaccines will require improved understanding of how influenza variants differ from each other. We find that temporal distances perform poorly overall, but even sequence distances which match phylogenetic distances well do not match cartographic distances based on actual immune response data.

Influenza A virus causes seasonal epidemics worldwide, primarily driven by continual evolution of the virus under selective pressure by host immunity [1]. Development of a universal influenza vaccine which can protect against novel strains of influenza has many challenges, including surveillance of new genomic variants and predicting which will be successful [2]. Statistical modeling and phylodynamic approaches are crucial tools in the development of a more broadly-protective influenza vaccine, but these methods rely on understanding how different each genomic variant of influenza actually is from its predecessors [3].

Many different metrics for assessing the antigenic difference between two influenza strains currently exist, including phylogenetic methods [4], sequence distances [5,6], and antigenic cartography, which is based on observed immunological data [7]. To understand the agreement in different distance measurements, we obtained data from a cohort study that has been previously described [8,9]. Using data from this study, we compared antigenic cartography and sequence methods to phylogenetic methods.

# Study Methodology

Briefly, our study data [8,9] consisted of volunteers enrolled at three different study sites from 2013 – 2019 who received a FluZone (Sanofi Pasteur) vaccine, and gave pre-vaccination and post-vaccination (21 or 28 day) serum samples. The serum samples were used for HAI assays against a panel of historical viruses. We computed the Hamming distance [5], -Epitope distance [6], and the absolute difference in the year of isolation of strains [10] from the sequences of all influenza viruses used for HAI assays, and used Racmacs to compute antigenic cartography distances from the HAI data [11]. All of our analyses were conducted separately for H1N1 and H3N2 strains.

In order to compare with phylogenetic methods, we first computed a multiple sequence alignment (MSA) using the MUSCLE algorithm [12]. The Hamming and -Epitope distances were computed based on this MSA. We had 18 H1N1 strains and 21 H3N2 strains in total. We then used both alignments to construct maximum likelihood (ML) unrooted phylogenetic trees using the FLU amino acid substitution model. We extracted the cophenetic distances between taxa from the ML trees, and compared these distances to our other distance metrics (temporal, Hamming, -Epitope, and cartography) using Pearson’s correlation.

For each of the four distance metrics, we also built distance-based trees using neighbor joining. To compare the methods, we calculated the likelihood of each of the distance-based trees, then estimated the Shimodaira-Hasegawa test statistic to compare each of the distance trees to the ML tree. Finally, we computed the Robinson-Foulds distance between each set of trees. Our analyses were implemented with R version 4.3.3 [13] using the packages phangorn [14] and msa [15].

# Study Results

We found that all four distance metrics were strongly correlated with cophenetic tree distance for H3N2, but for H1N1, only the Hamming and -Epitope distances had a strong correlation with the tree distance ([Figure 1](#fig-corr)). H1N1 has two clusters, 2009 pandemic-like (pdm) and non-pdm. The pdm-like strains are genetically more similar to the 1918 pandemic strain than to most strains which circulated from 1950 – 2009, so the temporal distance correlation is weak, as expected.

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| Figure 1: Scatterplots showing the cophenetic tree distance on the x-axis and the other distance metrics we calculated on the y-axes. The plots on the left are for H1N1 strains and the plots on the right are for H3N2 strains. The box shows Pearson’s correlation (R) along with a 95% Wald-type confidence interval. |

The cartographic distance correlation for H1N1 is also moderate, indicating that the evolutionary pattern of H1N1 strains does not necessarily explain variation in observed immune responses. For H3N2, the cartographic correlation was the lowest, and the two distances become less correlated as the distance values become larger. For closely related H3N2 strains the ability of the tree distance to predict differences in immune response appears to attenuate as strains drift further away.

The ML trees for both subtypes were able to reconstruct the patterns we expect for H1N1 and H3N2 influenza ([Figure 2](#fig-mltrees)). The H1N1 strains form two clades, one pdm-like clade which contains SC/18 (the 1918 pandemic strain), NJ/76 swine influenza, and the modern pdm-like strains. The other clade contains the H1N1 strains which circulated between the 1918 pandemic and the 2009 pandemic. The H3N2 strains tend to follow a similar ladder-like pattern, beginning with HK/68 and primarily separating by temporal distance, which corroborates the correlations between temporal and cophenetic distance ([Figure 1](#fig-corr)).

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| Figure 2: Maximum likelihood phylogenetic trees for H1N1 strains (left) and H3N2 strains (right). Both trees are rooted at the midpoint for display purposes, but the root was not optimized during fitting. |

For brevity, we do not show all 8 of the distance-based neighbor joining phylogenies. However, we conducted SH tests and computed the RF distance between each of the distance-based trees and the ML tree for the same subtype ([Table 1](#tbl-stats)). For the H1N1 strains, the temporal distance and cartographic distance trees were different from the maximum likelihood tree based on the SH test, and these trees also had a much higher RF distance from the ML tree than the Hamming and -Epitope distance trees. For the H3N2 strains, the -Epitope distance tree was different from the ML tree, and the cartographic tree was extremely different from the ML tree. The ML tree, temporal distance, and Hamming distance trees were all similar. All of the changes in log likelihood for the H3N2 trees were smaller in magnitude than for H1N1. Notably, the temporal distance tree had a much lower likelihood than the ML model for H1N1.

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| Table 1: Log likelihood of all constructed trees, along with the decrease in log likelihood (Δll) from the ML model, the p-value of the Shimodaira-Hasegawa test (SH p-value; evaluated on one million bootstrap resamples), and the Robinson-Foulds distance from the ML tree (RF distance).   |  | Tree | log likelihood | Δll | SH p-value | RF distance | | --- | --- | --- | --- | --- | --- | | H1N1 | Maximum Likelihood (baseline) | -3468.8 | 0.0 | N/A | 0 | | Temporal Distance | -5708.2 | 2239.4 | < 0.001 | 26 | | Hamming distance | -3469.4 | 0.6 | 0.875 | 2 | | p-Epitope distance | -3543.3 | 74.5 | 0.299 | 8 | | Cartographic distance | -3980.1 | 511.3 | < 0.001 | 24 | | H3N2 | Maximum Likelihood (baseline) | -3065.6 | 0.0 | N/A | 0 | | Temporal Distance | -3102.7 | 37.2 | 0.270 | 8 | | Hamming distance | -3110.2 | 44.6 | 0.214 | 4 | | p-Epitope distance | -3171.3 | 105.7 | 0.014 | 12 | | Cartographic distance | -3442.2 | 376.6 | < 0.001 | 30 | |

# Conclusions

Many papers still use the temporal method for calculating antigenic distance. However, for H1N1, the temporal distance completely fails to reconstruct any genetic changes. For H3N2, the temporal distance was similar to the ML distance.The Hamming and -Epitope distances were similar for both subtypes.

The cartographic distance tree was substantially different from the ML tree for both H1N1 and H3N2. Since cartographic distance is based on observed immune response data, this implies that the hemagglutinin sequence is not the only factor in determining individual immune responses. Our sample is likely not representative, so similar analyses should be repeated on other cohorts. Performing similar analyses using neuraminidase sequence and inhibition data would complete our findings well.

Overall we find that temporal methods should be avoided and are not suitable for calculating evolutionary distance between influenza strains. Additionally, the genetic distance between influenza strains does not match the cartographic difference from observed immune response data, indicating that genetic and antigenic evolution do not always agree.

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