DEVELOPMENT AND VALIDATION OF AN ALGORITHM PROGNOSTIC FOR INFLUENZA CONTAGIOUSNESS

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INTRODUCTION & OBJECTIVES

In an influenza pandemic, identifying individuals whom will subsequently become contagious before having significant contact with others would enable implementation of public health measures that could limit the spread of disease. The aim of this work was to develop and validate an algorithm prognostic for contagiousness using biomarkers identified at early time points post exposure to virus, before subjects would be exhibiting substantial clinical disease symptoms.

To facilitate identification of the prognostic biomarkers we utilised the influenza human viral challenge model (VCM) as it is an effective means of understanding a subject's pre-exposure biomarker levels and allowing precise monitoring of clinical disease and biomarkers throughout the infection time-course. There are numerous facets to contagiousness. Here we focus on the level of symptoms and viral shedding.

STRATEGIC APPROACH

Prognostic biomarkers were identified and algorithms developed during the discovery phase via 3 separate phenotype comparisons (**Fig 1**). Algorithms were subsequently then tested for contagious prognostic performance in a independent data set.

DISCOVERY PHASE / TRAINING SET
(90 subjects)

1. Infected subjects: high vs low virology
   - Upper tertile PCR vs Lower tertile PCR
2. Infected subjects: high vs low/no cold
   - Upper tertile VAS vs Lower tertile VAS
3. Infected subjects with low/no cold vs uninfected
   - Lower tertile VAS vs uninfected

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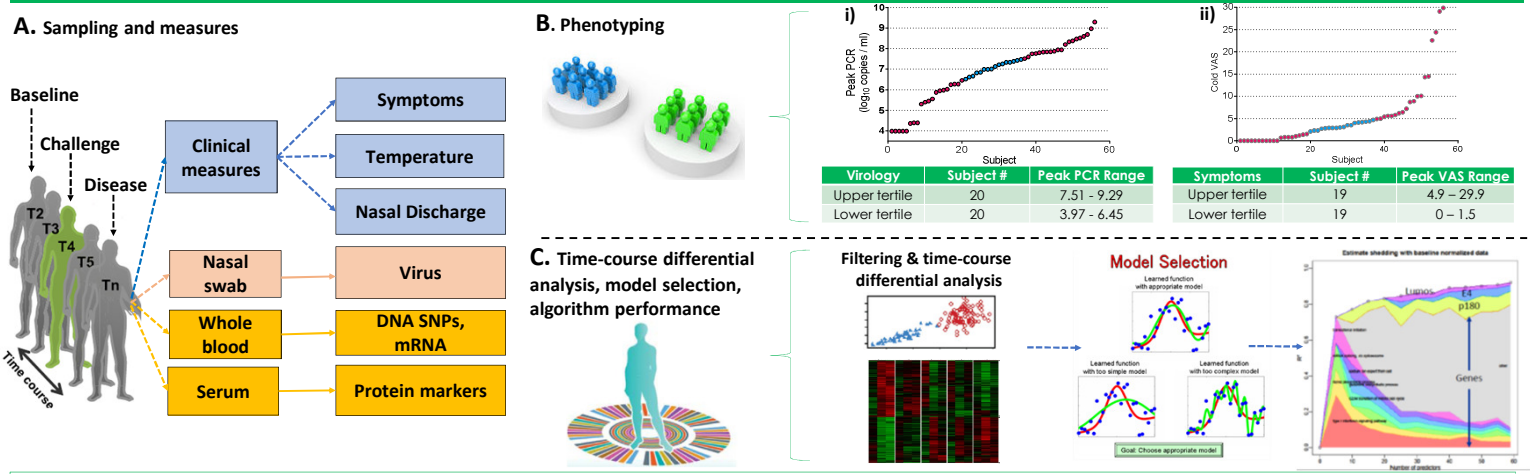
TESTING PHASE / INDEPENDENT TEST SET
(41 subjects)

Contagious vs Non-contagious

- Contagious = Upper tertile PCR and/or VAS
- Non-Contagious = remaining subjects

Fig. 1: phenotype comparisons used in both the discovery and testing phases. VAS: visual analogue scale for self reported influenza clinical symptoms

METHODS



Virology	Subject #	Peak PCR Range
Upper tertile	20	7.51 - 9.29
Lower tertile	20	3.97 - 6.45

Symptoms	Subject #	Peak VAS Range
Upper tertile	19	4.9 – 29.9
Lower tertile	19	0 – 1.5

Fig 2: Sampling and analytical pipeline. A) Clinical measures, nasal samples for virology, as well as frequent blood samples were taken for microarray transcriptomics and Luminex biomarkers. , B), Phenotype grouping by distribution tertiles: i) peak qPCR titre, ii) peak symptoms. Upper and lower tertiles shown graphically in red & mid tertile in blue. C) Differential expression analysis was performed, modeling time course omics data taking into account subject-subject variability. Profiles were identified that differ over time between phenotypes. Separate analysis was conduced on 0-24 hours and 0-60 hours time windows with the final time point in the window being used for testing (21 hour and 45 hour, respectively). Gradient boost modelling was applied and biomarkers ranked by relative influence. A parsimonious approach was taken to develop separate prognostic algorithms for both the microarray and Luminex platforms as well as pan-algorithms that combined data from the two analytical methods.

RESULTS

We identified numerous biomarkers that had differential time-course trajectories in subjects whom later became contagious from subjects whom did not. By utilising only those biomarkers with the greatest relative influence to minimise the risk of overfitting, we were able to develop several prognostic algorithms that showed good performance within the training set with AUCs from the ROC analysis in the 21 and 45-hour models ranging from 0.75 to 0.87 and 0.72 to 0.75, respectively (**Fig. 3**).

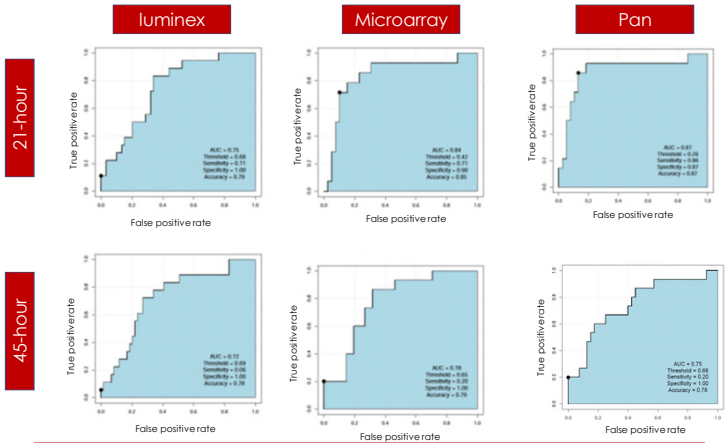


Fig. 3: Training data set ROC curve analysis. A separate 21-hour and 45-hour algorithm was developed and analysed within the training data set for both the Luminex and microarray platforms as well as a pan-algorithm combining both analytical methods. AUC, optimal threshold, sensitivity, specificity and accuracy results for each of the 6 algorithms are indicated on the respective ROC curve.

While the 21-model performed slightly better that the 45-hour modes in the training data set, all of the 21-hour models performed poorly in independent data-set testing. All the 45-hour models, however, performed well in their ability to correctly predict those individuals whom would subsequently become contagious, as indicated by ROC analysis AUC results ranging from 0.82 to-0.89 and optimal accuracy results of 0.85 to 0.88 (**Fig. 4**).

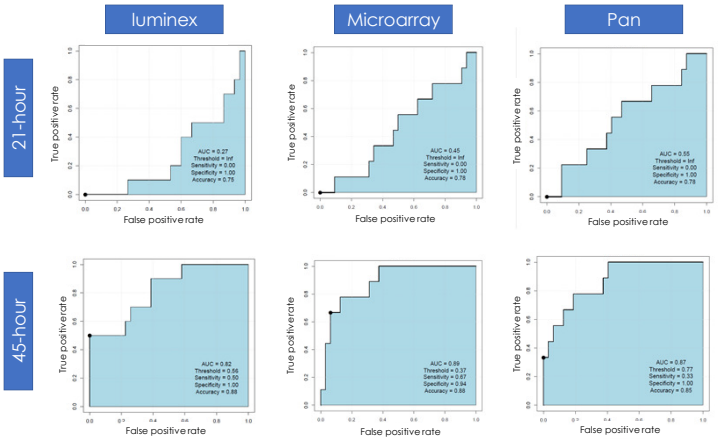


Fig. 4: Test data set ROC curve analysis. The 21-hour and 45-hour Luminex and microarray platforms as well as the pan-algorithm combining both analytical methods were tested for contagiousness prognostic performance in an independent test set. AUC, optimal threshold, sensitivity, specificity and accuracy results for each of the 6 algorithms are indicated on the respective ROC curve.

RESULTS SUMMARY

The 21-hour models did not perform well on independent data testing, however the 45-hour models demonstrated a high level of predictive performance both for the proteomic (Luminex) and transcriptomic (microarray) analytical platforms as well as the pan-algorithm that combined the two platforms. The independent test data results of the successful algorithms are summarised in the table below, as well as indicating the number of biomarker parameters utilised in each algorithm.

Algorithm platform	Number of parameters	ROC curve AUC	ROC curve optimal accuracy
Microarray	1	0.89	0.88
Luminex proteomics	2	0.82	0.88
Pan-algorithm	3	0.87	0.85

CONCLUSIONS

- We developed numerous potential prognostic algorithms that were tested on an independent data set.
- The results clearly indicate the importance of independent data-set testing, as not all algorithms retained good performance upon testing.
- 3 different algorithms were shown to retain good predictive performance and therefore passed the independent testing and were validated for contagious prediction and considered ready for field testing. This represents an important step forward in providing additional public heath tools for responding to influenza pandemics.
- Furthermore, this work demonstrates the utility of the human challenge model as a powerful tool with which to investigate host response of influenza infection.
- We postulate that the approach described here could also successfully be applied to other disease characteristics

ACKNOWLEDGEMENTS

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Figure 2: adapted and modified from Than & Snyder 2013 Review. iPOP Goes the World: Integrated Personalized Omics Profiling and the Road toward Improved Health Care. Cell Chemical Biology.