

# Correspondence

## Tracheostomy as a model for studying the systemic effects of local tissue injuries and the cytokine patterns of acute inflammation: design, rationale and analysis plan

Our current understanding of the connections between systemic responses to injury and their biochemical underpinnings is not well developed. It is based on the relationships between basic biological knowledge and clinical manifestations, which are mostly theoretical and based on statistical correlations. For instance, although the basic biology of tumour necrosis factor alpha (TNF $\alpha$ ) in inflammation is well known, we still don't know how TNF $\alpha$ -antagonists cause their beneficial clinical effects, why only some patients respond, or why the effect sometimes wanes over time<sup>1</sup>. There are several published trials trying to connect biochemical analytes with clinical responses<sup>2</sup>, yet they suffer from significant drawbacks and have provided few novel insights. We have yet to convincingly bridge the molecular and cellular responses to injury with their clinical effects on a systemic level<sup>3</sup>.

We have started a trial on bedside tracheostomies in order to further our knowledge on the connection between the biochemical processes and clinical presentations and outcomes. Tracheostomy represents a standard injury performed in relatively stable patients (therefore increasing the signal-to-noise ratio), and it provides an amenable platform for a meticulous study of the molecular and cellular response to injury. Although bedside tracheostomy is a common procedure and its outcomes have been well-studied in different populations, its systemic physiobiochemical effects haven't been studied<sup>4</sup>. The second aspect of our research is based on the application of a systems biology approach<sup>5</sup>. This approach combines the measurement of a large number of mediators representing different physiological pathways at several time points, integrating clinical measures and outcomes in the analysis; and, finally, the use of both statistical and bio-mathematical computational approaches to produce explanatory and predictive models.

In this study, detailed clinical and laboratory data will be collected from 14 days before and up to 28 days after tracheostomy. A large panel of 21 cytokines, chemokines and biomarkers will be measured from blood samples taken at five time points around the time of tracheostomy in a 24-hour period. Based on the observed standard deviations from our preliminary results, assuming 80% power and an  $\alpha$  error=0.05, 40 subjects are required to detect a twofold difference between different time points, which is expected according to the published literature. We have institutional permission (ethics approval number: 0220-14HMO) and an

initial goal of 50 subjects with the option for an increase to 100 patients. Mortality serves as the clearest, most tangible clinical outcome, with APACHE II and III as well as SOFA scores serving as secondary outcomes. C-reactive protein will serve as the main biochemical outcome. We will also analyse insulin requirements and enteral feeding rate as exploratory clinical outcomes. The clinical and molecular information collected will serve for developing statistical models of the correlations between these responses as well as with 28 day mortality. In addition, both the raw measurements and the statistical analyses will serve for validating a mechanistic model of the systemic effects of tracheostomy<sup>6</sup>.

This trial represents a collaborative effort, with different teams dealing with the clinical and experimental aspects, mathematical mechanistic modeling and statistical probabilistic modeling. We have so far recruited 29 patients. Preliminary analyses using parametric and non-parametric tests have yielded only a few barely statistically significant results. Yet direct observation of the raw data suggests novel signals and trends. For example, there are apparent clusters that could represent differing subtypes of cytokine expression responses between patients. All the laboratory analytes tested so far can be categorised into three to five clusters of response patterns. This might explain why measures of central trends that group all patients together are less likely to result in overall differences. Once more data are collected and these biochemical results are analysed together with the clinical measures and outcomes, we will be able to assess their significance and the validity of our approach.

In summary, our hypothesis is now that different mediators respond differently, different patients respond differently to the same stimulus, and these responses can be analysed and classified. We are currently at work recruiting more patients, performing the laboratory experiments and compiling the clinical data. In parallel, the mathematical and statistical teams are beginning to analyse the data available. Importantly, clinical data (and not just experimental data) will be included in building and validating the models and their predictions. The main expected result of the trial is a greater understanding of the connection between the biochemistry and clinical response to injury. Also, this study will hopefully expand our understanding of the consequences of tracheostomy and its applicability to different patient types and conditions. This project is also relevant for the field of personalised medicine. The ability to classify specific patient responses and then predict outcomes, biochemical and clinical, at the individual level, is one of the main drivers of our interest in this project. Indeed, if we wish to provide personalised treatments to our patients, the first step is to be able to relate their individual response patterns to clinical outcomes. In this project, we hope to demonstrate the feasibility of this approach. This trial represents the

initial step in a series of projects of increasing complexity and breadth by our collaborative research group, which we hope will help vertically integrate human physiology from biochemistry to clinical outcomes and help develop new diagnostic and therapeutic approaches.

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