ADJUVANT STEROIDS IN ADULTS WITH PANDEMIC INFLUENZA

Early low-dose steroids for adults admitted to hospital with influenza-like illness during a pandemic: a randomised placebo controlled trial

MECHANISTIC SUB-STUDY LABORATORY MANUAL

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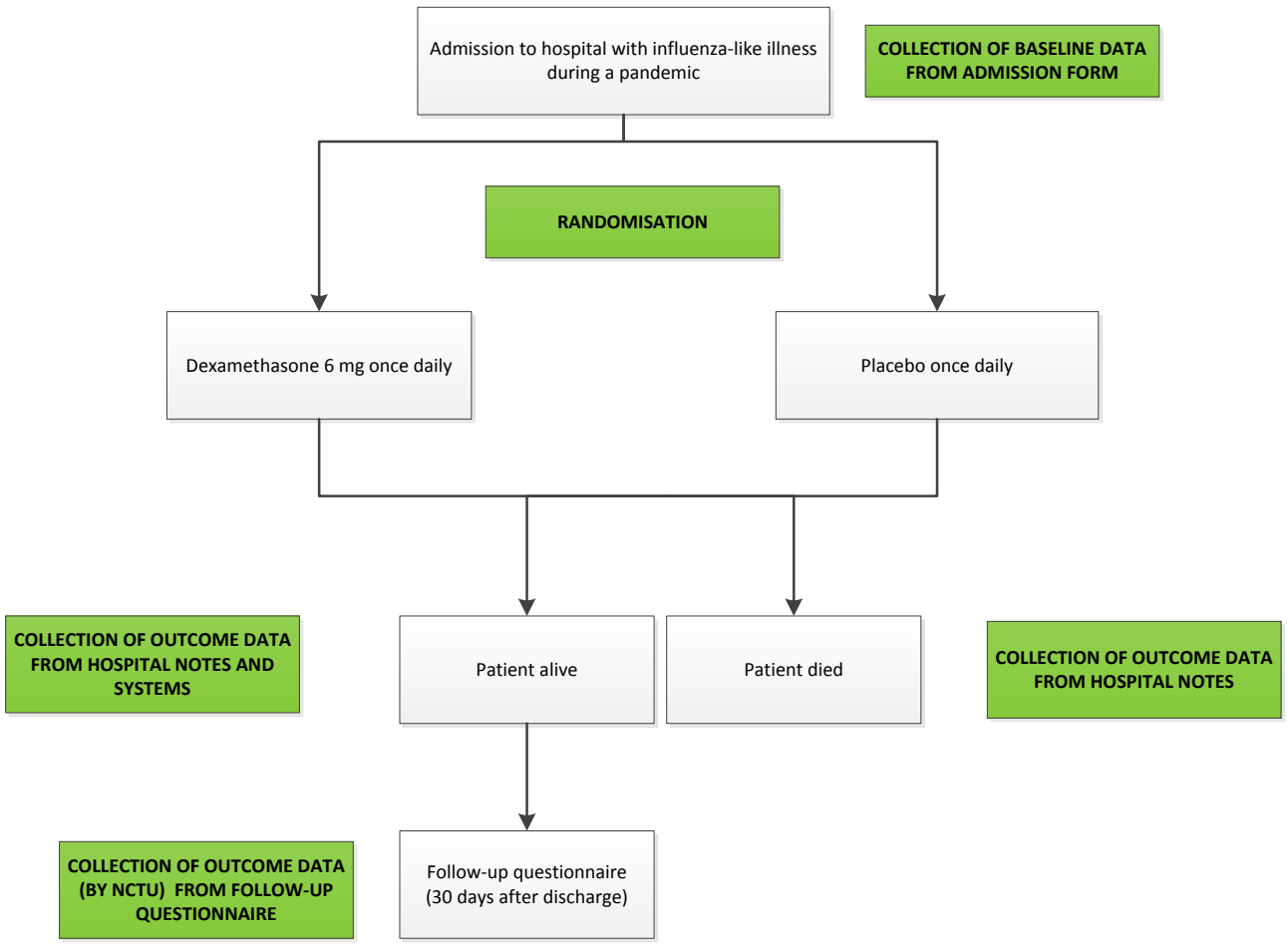
1 ABBREVIATIONS

Abbreviation	Definition
NCTU	Nottingham Clinical Trials Unit
EDTA	Ethylenediaminetetraacetic Acid
RT	Room Temperature
HTA	Human Tissue Authority
RNA	Ribonucleic Acid

2 TRIAL SUMMARY

Title	**Double-blinded randomised controlled trial of early low dose steroids in patients admitted to hospital with influenza during a pandemic**
Design	Randomised, placebo-controlled
Aims	To determine whether a 5-day course of dexamethasone, compared to placebo, started within 24 hours of admission to hospital, in addition to standard care is: 1) Associated with a lower risk of death or admission to intensive care compared to placebo **(PRIMARY OBJECTIVE)** 2) Associated with: a) a reduction in length of hospital stay b) the frequency of hospital readmission and/or the frequency of GP consultations after discharge **(SECONDARY OBJECTIVES)**
Intervention	• 15ml dexamethasone (6mg) once daily for 5 days started within 24 hours of admission to hospital (dose approximately equivalent to prednisolone 40 mg daily for 5 days) • 15ml placebo once daily for 5 days started within 24 hours of admission to hospital
Outcome measures	**PRIMARY** Admission to intensive care unit or death, within 30 days of admission **SECONDARY** 1. Length of stay in intensive care unit 2. Readmission within 30 days of hospital discharge 3. GP consultations within 30 days of hospital discharge 4. Length of stay in hospital 5. Death within 30 days of admission to hospital 6. Admission to intensive care unit within 30 days of admission to hospital The full statistical plan includes the flexibility to allow for pandemics of different severity.
Population	Adults (≥16 years old) hospitalised with an influenza-like illness during a pandemic.
Eligibility	• Aged ≥ 16 years • Admitted to hospital within the previous 24 hours with a clinical diagnosis of an influenza-like illness • Have given consent Exclusion criteria are: • Known to be taking oral or IV corticosteroid treatment • Require treatment with oral or IV corticosteroids upon admission to hospital as standard treatment for comorbid illness • Known to be on insulin or oral medication for the treatment of diabetes mellitus • Known contra-indication to dexamethasone or any of the excipients (refer to current version of SPC)
Duration	Recruitment is expected to last about 6 weeks.

3 TRIAL OVERVIEW



4 TRIAL ACTIVATION

Once the decision has been made to activate the ASAP trial, the NCTU will be in regular communication with sites regarding their activation; only once the geographical spread and severity of the pandemic are known will NCTU be able to clearly determine which sites will be opened to recruitment.

The NCTU will liaise closely will all trial sites regarding activation and recruitment during the pre-activation and activation phases of the trial.

5 MECHANISTIC SUB-STUDY

5.1 Aims

This mechanistic sub-study aims to address the following key questions in the treatment of influenza:

a) For each patient, what was the point in the natural history of influenza infection at which admission occurred?
b) What effect does steroid have on the natural history of influenza infection defined by clinical phenotype and RNA transcriptomic pattern?
c) Does comparison of the transcriptomic pattern in placebo versus steroid treated arm suggest pro-inflammatory and anti-inflammatory mechanisms are both altered?

5.2 Background

The NIHR have recommended that a mechanistic sub-study be conducted as part of the ASAP trial. This sub-study will be conducted in a limited number of pre-selected ASAP trial sites. These sites have been selected as they are considered to have sufficient staff and infrastructural capacity to manage the additional work related to the sub-study without compromise to clinical care delivery or conduct of the main ASAP trial.

The onset of symptoms is highly variable in influenza exposed adults[1] and the severity of symptoms is the result of a complex host-pathogen interaction[2] altered by co-infection and co-morbid illness[3]. Initially, cytokine data were used to describe this interaction, but recent data from a unique human influenza challenge study have fully described the human inflammatory and anti-inflammatory pathways that determine symptom severity in exposed adults[4]. Using whole genome arrays (Affymetrix Human Genome U133A v2), 5076 genes showed altered expression during the 5 days following influenza A exposure. Unsupervised and clinically informed analyses resulted in self-organising maps (clustered gene expression patterns) which showed 8 functionally important gene sets in which activation/inhibition was tightly associated with clinical severity (see reference 4). The study also described the duration in hours between virus exposure and significant difference in self-organising map (SOM) cluster expression between symptomatic/asymptomatic subjects.

Thus, this sub-study will apply the best contemporary (with respect to the time of pandemic declaration) methods to a representative sample (n=200) of ASAP trial participants in order to determine the interaction of steroid therapy and the host and to apply this in interpreting the clinical outcome measured.

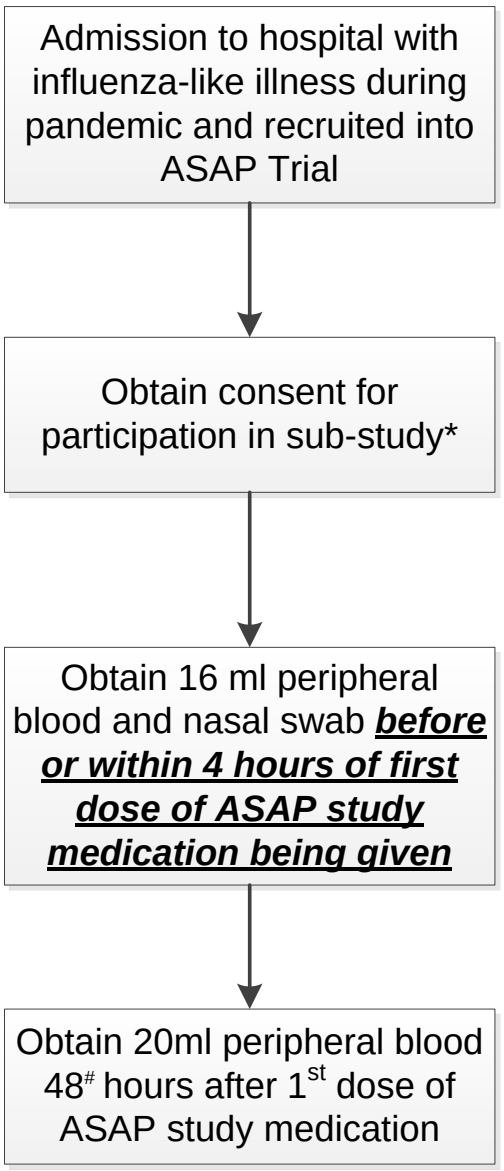
5.3 Methods/Design

Patients recruited into the ASAP trial at the sub-study sites will be given the opportunity to participate in the sub-study. 200 patients who have received ASAP study medication are required for the sub-study.

ASAP trial participants who consent to participation in the sub-study will give two blood samples and have one nasal swab taken for subsequent transcriptomic and microbiological testing. Blood samples will be collected into vacutainer tubes at baseline and 48 hours post first dose of ASAP study medication. These time points are practical and match the best available transcriptomic data and the expected time course of steroid effect on gene expression. These samples can be collected using standard venesection methods and transferred without urgency to frozen storage in the hospital clinical laboratories. These sample collections should therefore be minimally obstructive in the context of a pandemic. The nasal swab for virological confirmation will be obtained at the time of the first blood sample collection. Please see figure 1 for an overview of the sub-study design.

The overarching principle in relation to the conduct of the mechanistic sub-study is that it should not disadvantage or jeopardise conduct of or recruitment into the main ASAP trial. Six sites have been selected to participate in the sub-study and the decision to activate the sub-study at these sites will be determined by the Trial Management Group according to pre-agreed criteria. The ability of these sites to conduct the sub-study will continue to be monitored by the Trial Management Group throughout the recruitment phase.

Figure 1: Overview of the sub-study design



* Blood and nasal samples are required at the latest 4 hours after first dose of study medication so consent must have been obtained prior to the samples being taken.

Sample should be obtained at 48 hours (+/- 3 hours) or at discharge if this is sooner

6 SAMPLING REQUIREMENTS

Sub-study participants will give two blood samples and a nasal swab for sub-study purposes. All samples will be obtained by an appropriately trial trained doctor or nurse.

Table 1: Sub-study sample requirements

Time point	Sample	Details
Baseline (after recruitment into ASAP trial and before, or within 4 hours of 1st dose of ASAP study medication)	16 ml peripheral blood	2 x 2.5ml PAXgene tubes (RNA expression analysis) 1 x 6ml EDTA plasma gel tube (corticosteroid pharmacokinetics) 1 x 5ml serum gel tube (multiplex cytokine array)
	Nasal swab	Collected in standard viral transport medium. This sample will be in addition to any nasal swab taken for clinical purposes, but may be taken at the same time-point if appropriate.
Mid-treatment (48 hours (+/- 3 hours) after 1st dose of study medication, or prior to hospital discharge, whichever is sooner)	20 ml peripheral blood	1 x 5ml EDTA plasma gel tube (corticosteroid pharmacokinetics) 1 x 5ml serum gel tube (multiplex cytokine array) 1 x 10ml EDTA tube (genotyping)

7 SAMPLING PROCESSING AND STORAGE

7.1 Samples

At the time of enrolment (Time Point: Baseline), 16ml of peripheral blood will be sent to the laboratory in the tubes outlined above. A second 20ml peripheral blood sample must be obtained mid-treatment (Time Point: 48 hours (+/- 3 hours) after the first dose of ASAP study medication, or prior to hospital discharge, whichever is sooner).

7.1.1 Sample labelling

All aliquots and swabs should be labelled with the following information using **Label A** provided:

- **Participant Trial ID**
- **Sample Type (P = Plasma, S = Serum, PG = Pax Gene, NS = Nasal Swab, WB = Whole Blood)**
- **Time point (0h = baseline, 48h = 48 hour).**

ASAP sub-study sample
Participant ID: _____
P ☐ S ☐ PG ☐ NS ☐ WB ☐
0h ☐ 48h ☐

Label A

7.1.2 EDTA Plasma

- Centrifuge at 2500g for 10 minutes at room temperature.
- Transfer the plasma to a sterile container using a Pasteur pipette, taking care not to disturb the red cell layer.
- Split the plasma into pre-labelled (**Label A**) cryovials in 500µl aliquots (You should obtain approximately 5 to 6 aliquots from a 6ml sample [Baseline bloods] and approximately 4 to 5 aliquots from a 5ml sample [48 hour bloods]).
- Store all aliquots at -80°C until requested for transfer (ensure that the samples are logged on the electronic sample tracking system).

7.1.3 Serum

- Centrifuge at 2500g for 10 minutes at room temperature.
- Transfer the serum to a sterile container using a Pasteur pipette, taking care not to disturb the red cell layer.
- Split the serum into pre-labelled (**Label A**) cryovials in 500µl aliquots (You should obtain approximately 4 to 5 aliquots from a 5ml sample).
- Store all aliquots at -80°C until requested for transfer (ensure that the samples are logged on the electronic sample tracking system).

7.1.4 PAXgene

- PAXgene tubes must be left at room temperature for a minimum of 3 hours post-venepuncture (no longer than 72 hours) - The initial RT phase enables the RNA stabilisation solution to penetrate cells.
- Transfer PAXgene tubes to -21°C until frozen (allow 2 hours for freezing) - The -21°C freeze prevents the glass bottle cracking when taken down to -80°C.
- Once frozen, transfer PAXgene tubes to -80°C until requested for transfer (ensure that the samples are logged on the electronic sample tracking system).

7.1.5 EDTA Whole Blood (48 hour sample only)

- EDTA tube should arrive in the laboratory labelled with Participant Trial ID.
- Label sample with **Label A**.
- Store at -80°C until requested for transfer (ensure that the samples are logged on the electronic sample tracking system).

7.2 Nasal Swabs (baseline sample only)

- A nasal swab will be sent to the laboratory on enrolment (Time Point: Baseline). Swabs will be pre-labelled with **Label A**.
- Store at -80°C until requested for transfer (ensure that the samples are logged on the electronic sample tracking system).

All samples should be retained at site until recruitment to the sub-study has been completed.

7.3 Equipment for Sampling

All materials related to the mechanistic sub study samples will be purchased by, and packs created in, Liverpool. These study packs will then be sent out directly to the sub-study sites.

7.4 Storage Temperature Deviations

Any storage temperature deviations of +/-10°C should be reported to the NCTU. The laboratory should make appropriate provisions for the storage of trial samples in the event of a freezer failure.

8 SAMPLE TRANSFER

Frozen blood samples and nasal swabs should be sent by courier, on dry ice in a single batch to the University of Liverpool (UoL) where they will be stored at -80°C until analysed. A Material Transfer Agreement (MTA) will be established between recruiting sites and UoL to facilitate this.

The electronic sample log must be completed for all samples prior to dispatch.

9 SAMPLE ANALYSIS – UNIVERSITY OF LIVERPOOL

9.1 Blood samples

Once blood samples are received at the University of Liverpool, HTA compliant storage and archiving (Procuro system used for laboratory information) of blood samples will be provided in the University of Liverpool Respiratory Infection group.

Analysis of samples will be performed in a single batch using either RNA microarray expression analysis or next generation sequencing, depending on which is the most cost-effective method to obtain the data needed at the time analysis is carried out. This is a fast-moving technology and while the current balance would still favour microarray, it is anticipated that this will no longer be the case in a few years.

An inflammatory array of genes expressed during acute influenza (+/- steroids) will be described (using microarray or sequencing technology). These data will inform the clinical severity score, the duration of illness and most importantly will confirm the immune-modulatory effect of steroids

9.2 Nasal swabs (baseline sample only)

The latest test available to detect influenza virus including the pandemic strain will be used; this is expected to be a PCR test, however the specific test cannot be determined until the point of trial activation. After testing, all nasal swabs will be disposed of.

10 SURPLUS SAMPLES

Consent will be sought from sub-study participants for any remaining samples to be stored and used for future ethically approved research. Where consent has been given, remaining samples will be stored under a storage license from the Human Tissue Authority (HTA). Request for access to these samples for future ethically approved research will be made through the Chief Investigator and lead sub-study Investigator. Any outputs arising from use of these samples must reflect the contributions of both study teams.

11 DATA COLLECTION

No additional data over and above that collected for the ASAP trial is required for the sub-study.

12 WITHDRAWAL

Each sub-study participant has the right to withdraw from the sub-study at any time. In addition, the investigator may withdraw a participant from the sub-study at any time if the investigator considers it necessary for any reason. Any samples already collected for the sub-study would still be used unless specifically requested not to, in which case any samples collected would be destroyed.

The reason for withdrawal from the sub-study will be requested and recorded in the trial database; however participants are not obliged to give reasons. There will be no replacement of participants who withdraw from the sub-study.

13 STATISTICAL ANALYSES

Data arising from the sub-study will be analysed by sub-study investigators at the University of Liverpool and University Hospital Aintree.

The following assumes that the platform chosen to achieve the stated aims is microarray analysis of the whole blood RNA transcriptome between subjects (placebo vs control) normalised to the transcriptome of a set of healthy volunteers.

All analyses will be conducted in the latest version of R (currently 3.1.0). For analysis of the RNA transcriptome data we will use the latest version of the package Bioconductor (currently version 2.14). An initial normalisation step will be carried out to account for technical variation in the gene expression data between samples. Differential gene expression analysis will be explored between treatment and control samples using the normalised expression data.

- To address aim one, using day zero data, levels of expression of genes that defined temporal stage in the paper by Huang et al. will be summarised using appropriate descriptive statistics in order to define when in the course of flu infection patients were recruited to the study.

- To address aim two comparing steroid with placebo groups, between-group differences in the expression of relevant genes on day 3 will be estimated using appropriate regression models 4.

- To address aim three - functional analysis of genes of interest (those that are differentially expressed between treatment and placebo groups) will be performed to infer the mechanism of action of steroid when given to patients with pandemic influenza; this will be a descriptive analysis.

14 REFERENCES

1 F. Carrat, E. Vergu, N. M. Ferguson, M. Lemaitre, S. Cauchemez, S. Leach, and A. J. Valleron, 'Time Lines of Infection and Disease in Human Influenza: A Review of Volunteer Challenge Studies', *Am J Epidemiol,* 167 (2008), 775-85.

2 M. D. de Jong, C. P. Simmons, T. T. Thanh, V. M. Hien, G. J. Smith, T. N. Chau, D. M. Hoang, N. V. Chau, T. H. Khanh, V. C. Dong, P. T. Qui, B. V. Cam, Q. Ha do, Y. Guan, J. S. Peiris, N. T. Chinh, T. T. Hien, and J. Farrar, 'Fatal Outcome of Human Influenza a (H5n1) Is Associated with High Viral Load and Hypercytokinemia', *Nat Med,* 12 (2006), 1203-7.

3 M. B. Rothberg, and S. D. Haessler, 'Complications of Seasonal and Pandemic Influenza', *Crit Care Med,* 38 (2010), e91-7.

4. Y. Huang, A. K. Zaas, A. Rao, N. Dobigeon, P. J. Woolf, T. Veldman, N. C. Oien, M. T. McClain, J. B. Varkey, B. Nicholson, L. Carin, S. Kingsmore, C. W. Woods, G. S. Ginsburg, and A. O. Hero, 3rd, 'Temporal Dynamics of Host Molecular Responses Differentiate Symptomatic and Asymptomatic Influenza a Infection', *PLoS Genet,* 7 (2011), e1002234