Prospective observational study in patients with obstructive lung disease: NOVELTY design

Online supplement

Methods

Due to the observational nature of the study, most clinical assessments will be conducted according to the patient's usual standard of care, as per local guidelines. The exceptions are the performance of spirometry, the measurement of fractional exhaled nitric oxide (FeNO) and the collection, storage and shipment of biosamples, which will be performed in accordance with standardised procedures, summarised below.

Spirometry

Spirometry will be performed locally by site personnel according to American Thoracic Society/European Respiratory Society (ATS/ERS) criteria [1]. Sites that do not have suitable spirometry equipment will be provided with a spirometer for central recording of spirometry data. For sites that will use their own spirometry equipment, the equipment must comply with the ATS/ERS criteria. All sites will receive basic spirometry training. At sites provided with a spirometer, site staff performing spirometry will also receive training on the spirometry device and will undergo a proficiency test (spirometry data will be centrally checked for quality). At all sites, a proportion of baseline spirometry data will be centrally reviewed to check quality. Prior to reversibility testing at the baseline visit, patients should withhold their short acting bronchodilator medication(s) for at least 6 hours and long-acting bronchodilators (with or without ICS) for 12–24 hours, depending on the medication. Post-bronchodilator spirometry should be performed a minimum of 15 minutes after taking the bronchodilator.

FeNO

FeNO will be measured locally by site personnel according to the recommendation of the equipment manufacturer and the ATS/ERS recommendations [2]. Sites that do not have suitable FeNO equipment will be provided with a Niox Vero device (Circassia, Oxford, UK) for the duration of the study. For sites that will use their own FeNO device, the device must comply with the ATS/ERS guidelines and be CE marked or 510(k) approved. All sites will receive basic FeNO training. At sites provided with a FeNO device, site staff performing FeNO will also receive training on the FeNO device.

Collection, storage and shipment of biosamples

Site staff will be provided with, and trained from, a detailed study-specific Central Laboratory Services Manual prepared by Covance Inc. (Princeton, NJ, USA). Procedures are summarised below.

Whole blood collection for haematology and differential blood count

Whole blood for haematology and differential blood count will be collected into an EDTA tube. Blood smears will be made at the time of collection for confirmation of haematology results. Samples will be shipped at ambient temperature on the day of collection.

Haematology and differential blood count analytes

- Haematocrit
- Haemoglobin
- Mean corpuscular haemoglobin
- Mean corpuscular haemoglobin concentration
- Red blood cells
- Red blood cell morphology and mean corpuscular volume

- White blood cells
- Basophils (absolute and percentage)
- Eosinophils (absolute and percentage)
- Lymphocytes (absolute and percentage)
- Monocytes (absolute and percentage)
- Neutrophils (absolute and percentage)
- Platelets

Blood collection for serum biomarkers

Blood for serum biomarkers will be collected into a tube without gel and thoroughly mixed with the clotting agent, then allowed to clot for 30 min standing upright, followed by centrifugation at 1500–2000 x *g* for 15 min. Serum will be transferred to a separate tube and frozen immediately at or below −20°C, then shipped frozen on dry ice on the day of collection.

Blood collection for plasma biomarkers

Blood for plasma biomarkers will be collected into a lithium heparin tube and immediately mixed by gently inverting at least 8 times, followed by centrifugation at 1500–2000 x *g* for 15 min. Plasma will be transferred to a separate tube and frozen immediately at or below −20°C, then shipped frozen on dry ice on the day of collection.

Urine collection

Urine will be collected into sterile collection cups. The sample will be transferred to tubes and frozen immediately at or below −20°C, then shipped frozen on dry ice on the day of collection.

Whole blood collection for RNA analysis

Whole blood for RNA analysis will be collected into a room temperature (18–25°C) PAXgene tube, after priming the interior volume of the blood collection set and taking care to prevent backflow. The sample will be gently mixed and stored upright at room temperature (18–25°C) for 2–3 hours before transferring to −20°C, then shipped frozen on dry ice (with care taken to ensure tubes are buffered from direct contact with dry ice) on the day of collection.

Whole blood collection for DNA analysis

Whole blood for DNA analysis will be collected into an EDTA tube. Samples will be gently mixed and frozen immediately at or below −20°C, then shipped frozen on dry ice on the day of collection.

DNA extraction from whole blood

DNA will be extracted from the whole blood sample using the following method:

(1) Mix whole blood with lysis buffer and centrifuge to pellet the white cells. Resuspend in red cell lysis buffer and repeat centrifugation.

(2) Lyse white blood cells and deproteinate with sodium perchlorate.

(3) Extract DNA with chloroform and Nucleon resin and precipitate DNA with ethanol, centrifuge and wash with ethanol.

(4) Dry DNA pellet and suspend in Tris/EDTA buffer for storage at −70°C.

Biobanking procedures

Where possible, biosamples will be aliquoted at collection to avoid freeze-thaw cycles, and stored at −70°C upon reaching the central repository. For example, for serum and plasma

samples, 5 aliquots will be made of each sample, with a maximum volume of 1 mL per aliquot for serum samples and 200 µL per aliquot for plasma samples.

Biosamples will be labelled and shipped in accordance with the Laboratory Manual and the Biological Substance, Category B regulations, according to the International Airline Transportation Association (IATA) 6.2 guidance document. A full chain of custody will be maintained for all samples.

Biosamples (with the exception of extracted DNA samples) will be stored in the central repository in Gothenburg, Sweden and held for batched analysis. Extracted DNA samples will be stored either at the central repository in Gothenburg, Sweden (for Swedish samples) or at Fisher Genetic Biobank, UK (for all other samples).

Biosamples will be retained for a maximum of 15 years after the finalisation of the clinical study report, and coded to maintain patient confidentiality. Each sample will have full traceability, through the Principal Investigator at each site and the sample receiver in the central repository. Therefore, any patient who withdraws consent will have their samples destroyed, if not already analysed and documented.

References

1. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J. Standardisation of spirometry. Eur Respir J 2005; 26: 319-338.

2. American Thoracic Society and European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. Am J Respir Crit Care Med 2005; 171: 912-930.