Statistical Analysis Plan – Analysis 1

Best Available Treatment Study

Introduction
This statistical analysis plan builds upon the general principles and specifics of the preliminary analysis plan in the study handbook.

Data preparation
Data are entered in RedCap version 6.14.2. Included patients will be finalised on 24 February 2021, with subsequent data changes restricted to correction of errors and missing data. Subsequent processing and analysis will be undertaken in R version 4.0.2, using the packages WeightIt, Cobalt and Survey. Validation and correction of admission, discharge and immunomodulatory treatment dates will be undertaken. Data will be processed such that repeated clinical, laboratory and treatment variables are represented in a table with one row per patient-day.

Exclusions

Patients will be excluded from analysis if an admission date is unavailable, data is not entered on the treatment form, there is no daily data and no discharge date or the date of first immunomodulatory treatment was unclear.

Only patients treated from the day of admission or transfer will contribute outcomes for weighted analyses. Death, complications and secondary diagnoses can be reported unadjusted on patients including those treated in the days before transfer.

Missing data and interpolation

Level of care variables, including respiratory support and inotropes, and the clinical variable fever will be interpolated for missing daily data where preceding and following values are identical. Where missing data for respiratory support and inotropes follow a final value, if the final value indicates no support was needed, subsequent daily values will be considered to be the same.

Further, where total number of days of invasive ventilation, non-invasive ventilation, oxygen and inotropic support are available, missing data will be entered assuming no discontinuous periods of treatment (preliminary analysis shows a low frequency of multiple episodes of inotropes, ventilation or oxygen usage in complete data).

Merging consecutive admissions

Where multiple hospitals within one location report patients, we will inspect plots of admissions and ages to identify possible consecutive admissions. More detailed comparison of age, gender, weight, admission periods and laboratory and clinical variables will be used to confirm. Consecutive admissions will be merged into a single record by splicing daily data and taking initial admission baseline data and final admission outcomes.

Laboratory values

Each site will report laboratory variables in units prespecified in the data collection tool, or with alternative units. Conversion to the same units will be undertaken. Manual inspection of result distributions from individual sites will be used to identify and correct incorrect or discrepant units. Extreme outliers will be inspected on a per individual basis and corrected when the value is discrepant with the rest of the biomarker time course. Extreme outliers are those visibly far outside the range of most results.

Clinical severity scale

For each day of admission, clinical severity will be calculated on an ordinal scale:

1. Death
2. Extra-corporeal membrane oxygenation
3. Ventilated (invasive or non-invasive) and on inotropic support
4. Ventilated (invasive or non-invasive)
5. Inotropic support
6. Receiving oxygen
7. No supportive therapy CRP ≥ 50
8. No supportive therapy Unknown
9. No supportive therapy CRP < 50
10. Discharged
11. Transferred

Levels 3-10 will be considered for clinical improvement outcomes. The additional levels will aid in graphical presentation.

Demographics and baseline clinical data

Age is collected in years and additional months. Where additional months are missing they will be assumed to be zero. If age in years is missing and the data cannot be obtained, the child's age will be replaced with the median age in the cohort.

Patients' weight-for-age Z scores will be calculated from the WHO reference data using the RCPCH Growth API. The World Bank lending group classification will be used for country economic status.

Significant past medical history will be regarded as primary or secondary immunodeficiency, HIV, autoimmune disease, chronic lung disease, chronic neurological disorder or malignancy.

Treatment definitions

Patients will be grouped according to first treatments received on the same calendar day. Those receiving IVIG alone, steroids alone or IVIG and steroids in combination, will be selected for comparison in this first analysis as these are accumulating the greatest proportion of patients. IVIG alone will be taken as the reference category.

Primary outcome definitions

Inotropic support, ventilation and death (dichotomous)

Inotropic support and ventilation (invasive or non-invasive) at any time from the second day post-treatment, or death at any time. Inotropic support and ventilation will be regarded as not available if the patient was transferred or died on day one or two, without report of support being received on day 2. If the patient was discharged on day 1 or 2, the outcome will be regarded as negative. Death will be regarded as missing for all transferred patients, and as negative for all patients whose destination was not recorded.

Improvement at day 2 (dichotomous)

Improvement at day 2 will be reported relative to day 0 for:

- Any patient who was discharged on or before day 2
- Patients stepped down from ventilation or inotropic support
- Patients not ventilated or on inotropes who stepped down from oxygen
- Patients not receiving organ support whose CRP fell from above 50 mg/l on or before the day of treatment to below 50 mg/l.

Improvement will be regarded as unknown if a patient was transferred on or before day 2, and negative for a patient who died on or before day 2.

Sensitivity analyses

Two planned sensitivity analyses will be undertaken:

- Patients fully meeting the WHO criteria for MIS-C
- Defining primary treatment as all immunomodulatory treatments administered over two consecutive days (day 0-1)

Additional sensitivity analyses described in the study handbook are preserved for future analyses with larger cohorts.

Subgroup analyses

No subgroup analyses are planned for this analysis, though *post-hoc* exploratory analyses may be undertaken.

Correction for multiple testing

This will be undertaken using the Bonferroni-Holm method for the two primary outcomes

Secondary outcomes definitions

Failure/escalation of primary treatment

Defined as the addition of any immunomodulator from the first day after primary treatment. For patients receiving corticosteroids within primary treatment, an escalation of more than 5 mg/kg prednisolone equivalent in total daily dose will be required for further steroid usage to class as failure. If transferred before the fifth day following primary treatment, failure will be regarded as not available.

Time to improvement in clinical severity

For each patient the time to improvement in clinical severity was calculated as:

- Time to come off ventilator or inotropes for patients receiving both therapies

- Time to come off ventilator for patients ventilated
- Time to come off inotropes for patients receiving inotropes
- Time to come off oxygen for patients receiving oxygen
- Time for CRP to fall below 50 mg/l for patients with final CRP on day of treatment or earlier of greater than or equal to 50 mg/l
- Time until discharge for all patients, where other event did not precede
- Time to come off ventilator

Death

As defined in composite primary outcome.

Fever

Presence of fever at any point from day 2. If no fever reported, but missing data, the outcome will be regarded as not available.

Increase in level of support:

This was based on any commencement of:

- ECMO for patients not on ECMO on day 0
- Ventilation for patients not ventilated on day 0
- Inotropic support for patients not ventilated on day 0
- Oxygen for patients not on oxygen on day 0

Where none of the above led to classification of deterioration, death was regarded as deterioration and transfer was regarded as the outcome being unavailable. Patients discharged home or with unreported discharge destination were regarded as not having increased support.

Persisting coronary artery dilatation

The presence of a coronary artery with Lopez z-score ≥ 2.5 or a report of aneurysm without z-score on the final echocardiogram, undertaken on the second or subsequent days following treatment. Will be regarded as not available if no echocardiogram reported, and negative if echocardiogram reported with no aneurysm or z-score ≥ 2.5. Presence of pre-treatment coronary artery dilatation will be added as a balancing covariate.

Inflammatory markers

Inflammatory markers will be plotted as percentages of the peak value, per patient, throughout the course of their admission. Line plots will be weighted by covariate-balancing propensity scores as described below. Smoothed curves with confidence intervals will be plotted using a generalized additive model (geom_smooth from the ggplot2 package in R).

Complications of drug therapy

Complications deemed by the treating clinician to be the result of immunomodulatory treatment, including but not limited to: allergy/anaphylaxis, cataracts, gastric perforation, gastric ulceration, hip necrosis, hyperglycaemia, hyperlactataemia, opportunistic infection, profound bradycardia, psychosis and steroid-induced hypertension. These will be reported descriptively.

Left ventricular dysfunction

The presence of left ventricular dysfunction on any echocardiogram 24 hours after commencement of primary immunomodulatory treatment. For this analysis, the presence of left ventricular dysfunction prior to starting immunomodulatory treatment will be added as an additional covariate for calculation of propensity scores to control for confounding due to potential differences in pre-treatment prevalence in each of the treatment arms.

Study enrolment Data

A descriptive analysis will be performed to summarize the number of countries and sites within each country that have enrolled onto the study, along with the number of patients from each country and site. Monthly enrolment numbers will be reviewed to show uptake and study progress.

Analysis

Descriptive analyses

Demographic data

Demographic data will be summarized in a table, including: gender (proportion male/female), age (mean and standard deviation), ethnicity, weight for age (z-scores), proportion with significant comorbidities, and country classification by income level (as defined by the World Bank classification). In addition to presenting the data for the population as a whole, further stratification will be done by diagnostic groups. This will include patients not have all the WHO criteria, and patients excluded from WHO criteria due to bactereamia or reported toxic shock syndrome.

Clinical features and laboratory markers

Salient clinical features during the patient's admission will be summarized and tabulated, including: fever, sore throat, cough, respiratory distress, abdominal pain, diarrhea, vomiting, headache, encephalopathy, irritability, lethargy, and SARS-CoV-2 PCR status. We further present Kawasaki Disease features during admission including the presence of: rash, red lips, mucosal membrane changes, conjunctivitis, oedema, skin peeling, lymphadenopathy, and BCG reactivity.

Laboratory markers are summarized and tabulated showing mean and standard deviation and include: CRP, troponin, lactate dehydrogenase, D-Dimer, ferritin, white cell count, lymphocyte count, neutrophil count, platelets, creatinine, BNP, haemoglobin, prothrombin time, activated partial thromboplastin time, and fibrinogen. These clinical and laboratory marker data will be presented for the entire cohort and further stratified by diagnostic groups.

Confounding

All primary outcomes, sensitivity analyses, and secondary outcomes (excluding death, secondary diagnoses and complications) will undergo analysis following weighting by multinomial covariate-balanced propensity scores to control for baseline confounding factors, as implemented by WeightIt version 0.11.0, using the "just-identified" approach. The Average Treatment Effect (ATE) will be estimated, except when comparing inflammatory markers between treated and untreated patients, when the Average Treatment Effect in the

Treated (ATT) will be calculated with the untreated group as the reference due to the likely dissimilarity of a smaller untreated group and the need to preserve the full sample.

The following variables will be considered for balancing:

1. Transfer vs. admission (dichotomous)
2. Treated in referring hospital (dichotomous)
3. Age (continuous)
4. Sex (binary)
5. Weight-for-age z-score greater than 2 (binary with missingness indicator)
6. Significant comorbidity (binary)
7. Days since fever at admission (continuous with missingness indicator)
8. Days of admission at treatment (continuous)
9. Total number of important clinical features reported up to day 0 (continuous)
10. COVID status: PCR positive, serology positive (if not PCR positive) or no positive result
11. Peak clinical severity to day of treatment (categorical)
12. Direction of change in clinical severity at day of treatment: increasing, stable, decreasing or unavailable (categorical)
13. Peak CRP up to day of treatment (quartile, or missing)
14. Direction of change in CRP at day of treatment (increasing, decreasing or unavailable)
15. Peak troponin up to day of treatment (quartile, or missing)
16. Peak BNP up to day of treatment (quartile, or missing)
17. Peak D-dimer up to day of treatment (quartile, or missing)
18. Coronary artery status up to day of treatment: last Z score ≥ 2.5, last Z score < 2.5, or not available

This will be reduced based on data availability and clinical priority as determinants of treatment and outcome. Important covariates will be added for certain secondary analyses as described above.

Balancing will be repeated for every analysis on the population providing the outcome. No imputation for missing outcome data will be undertaken.

We will aim for absolute standardised mean differences of 0.1 in continuous variables, and below, and Kolmogorov-Smirnov distances of 0.1 and below. Love plots will be used to examine the extent of imbalance and consider the potential impact. We will tolerate some deviation since covariates are also included in outcome models.

Models

Modelling approaches producing robust sandwich standard errors will be used, with dichotomous outcomes to be analysed using the survey package, adding all covariates used in covariate balancing, to produce doubly-robust estimates. Generalised linear models with a quasibinomial link function will be used to estimate odds ratios and 95% confidence intervals.

Time to event analyses will be undertaken using weighted Cox proportional hazards model[1] estimated average hazard ratios. This allows for violation of the proportional hazards assumption.

Clinical severity over time

Clinical severity over time will be presented as proportional column charts from two days before treatment to 10 days after treatment. Only patients treated after day 1 will contribute severity data for preceding days, since patients treated on day 1 of admission will provide no severity data for pre-treatment days. The charts will be presented both unadjusted and weighted by the covariate-balanced propensity score.

References

1. Schemper, M., Wakounig, S. & Heinze, G. The estimation of average hazard ratios by weighted Cox regression. *Stat. Med.* **28**, 2473–2489 (2009).