HYPO ECMO Statistical analysis plan
11/01/2021
Validated before database lock
K Duarte / N Girerd

Study endpoints

Primary endpoint

All-cause mortality at day 30 will be the primary outcome variable of this analysis.

Secondary endpoints

Secondary endpoints will be tested in a hierarchical fashion [1] as listed in the following Table:

Table 1: Secondary endpoints and hierarchical testing order

Hierarchical Testing Order	Secondary Endpoint
1	Composite endpoint of death, cardiac transplant, escalation to LVAD, stroke at day 30
2	All-cause mortality at day 180
3	Composite endpoint of death, cardiac transplant, escalation to LVAD, stroke at day 180
4	All-cause mortality at day 60
5	Composite endpoint of death, cardiac transplant, escalation to LVAD, stroke at day 60
6	All-cause mortality at day 7
7	All-cause mortality at 48 hours
8	VA-ECMO duration
9	Cumulated amount of administered fluids
10	Duration of vasopressors use in ICU
11	Duration to normalization of lactate
12	Number of days alive without organ failure(s), defined with the SOFA score and its components (respiratory, liver, coagulation and renal), between inclusion and day 7
13	Number of days alive without organ failure(s), defined with the SOFA score and its components (respiratory, liver, coagulation and renal), between inclusion and day 30
14	Duration of mechanical ventilation
15	Number of days of mechanical ventilation between inclusion and day 30
16	Number of days of mechanical ventilation between inclusion and day 60
17	Number of days of mechanical ventilation between inclusion and day 180
18	Duration of ICU stay
19	Duration of hospitalization
20	Number of severe and moderate bleeding complications estimated using the BARC classification under VA-ECMO
21	Number of packed red blood cells transfused under VA-ECMO
22	Pulmonary infections
23	Blood infections
24	VA-ECMO cannulaes infections
25	Lactate clearance at day 1
26	Lactate clearance at day 2
27	Lactate clearance at day 3
28	Lactate clearance at day 4
29	Lactate clearance at day 5

Hierarchical Testing Order	Secondary Endpoint
30	Lactate clearance at day 6
31	Lactate clearance at day 7

Statistical analysis

Sample size consideration
We anticipated that mortality in cardiogenic patients supported with VA-ECMO will be 50% (based both on ELSO data, Combes data (Crit Care Med. 2008 May;36(5):1404–11), as well as personal data from the study's principal investigator (database of 150 patients)). Considering a total event risk (for the primary endpoint) of 50% in the control group, a sample size of n=167 patients/group will detect a 15% absolute difference in favour of the VA-ECMO group using a chi-square test with an 80% power and considering a two-sided global alpha level of 5% using the LanDe Mets method with O'Brien-Fleming boundary for one interim analysis after inclusion of two-third of the patients.

General considerations
All analyses will be performed using SAS software version 9.4 (SAS Institute, Cary, NC, USA) and R software version 4.0.3 (the R foundation for Statistical Computing).

Continuous variables
Continuous variables will be described by the number of non-missing values, mean and standard deviation. The normality of the distributions will be assessed. In case of violation of normality, the continuous variables will be described by the median and interquartile range (1^{st} quartile and 3^{rd} quartile).

Categorical variables
Categorical variables will be summarized by the observed frequencies and the percentages relative to the total number of non-missing items.

Description of statistical analysis of primary and secondary endpoints
As per intention to treat analysis, every randomized patient will be included in the analysis of primary and secondary endpoints.

Analysis of the primary endpoint
The differences between the two study groups (ie, intervention and controls) relative to the risk of all-cause mortality at day 30 will be studied using logistic regression adjusted for the postcardiotomy setting, prior cardiac arrest, prior MI, age, vasopressor dose, SOFA score and lactate at randomisation. Resulting associations will be illustrated by means of Kaplan-Meier survival curves, and a similarly adjusted HR will also be provided as well as crude estimates of ORs and HRs.

One interim analysis for efficacy/futility was performed after inclusion of 2/3 of the patients. To maintain an overall type I error rate of 5% (two-sided), a bilateral p-value lower than 4.9% will be considered significant for the final analysis.

For the primary outcome, we do not expect missing variables. All-cause 30 day mortality is a straightforward outcome that does not require detailed information. Yet, in the unlikely case of missing vital status at 30 days, patients with missing data will be analyzed with the last observation being carried forward. In a sensitivity analysis, the worst case scenario method will be used (i.e. all patients with incomplete follow-up data died in the intervention group and died in the no intervention group).

An interaction analysis will be performed using the following list of possible predictive markers: postcardiotomy setting, prior cardiac arrest, prior MI, age, vasopressor dose, SOFA score and lactate at randomisation.

Analysis of the secondary endpoints

Secondary endpoints will be tested in a hierarchical fashion and are listed in Table 1 (page 1). A hierarchical testing procedure will be implemented in the order shown in this table until the p-value exceeded 0.049 [1].

For the secondary analysis of a) all-cause mortality at 48 hours and at days 7, 60, 180, and b) the composite endpoint of death, cardiac transplant, escalation to LVAD, stroke at days 30, 60, 180, the same analysis strategy will be performed as for the primary endpoint.

An unpaired t-test will be performed, after testing of the variables for normality of distribution of continuous outcomes. Linear regression adjusted for the same variables as those depicted in the primary endpoint analysis will also be performed. Importantly, in case of non-normal distributions, non-parametric tests will be used.

Chi-square tests (or Fisher's exact test in the event of an insufficient number of expected patients in the 2×2 cell) will be performed for categorical outcomes other than those described above and odd-ratios will be provided for illustrative purposes.

Reference

[1] Harrington, D. et al. New guidelines for statistical reporting in the journal. N. Engl. J. Med. 381, 286 (2019).