Supplementary Material for analysis of Anti-Xa and APTTr anticoagulation on VV-ECMO patients

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1 VARIABLES and DEFINITIONS

1.1 Outcome variables

1. Bleeding or thrombotic events(BTE)

1a. no BTE - 1b. only haemorrhagic complications 1c. only thrombotic complications 1d. both BTE

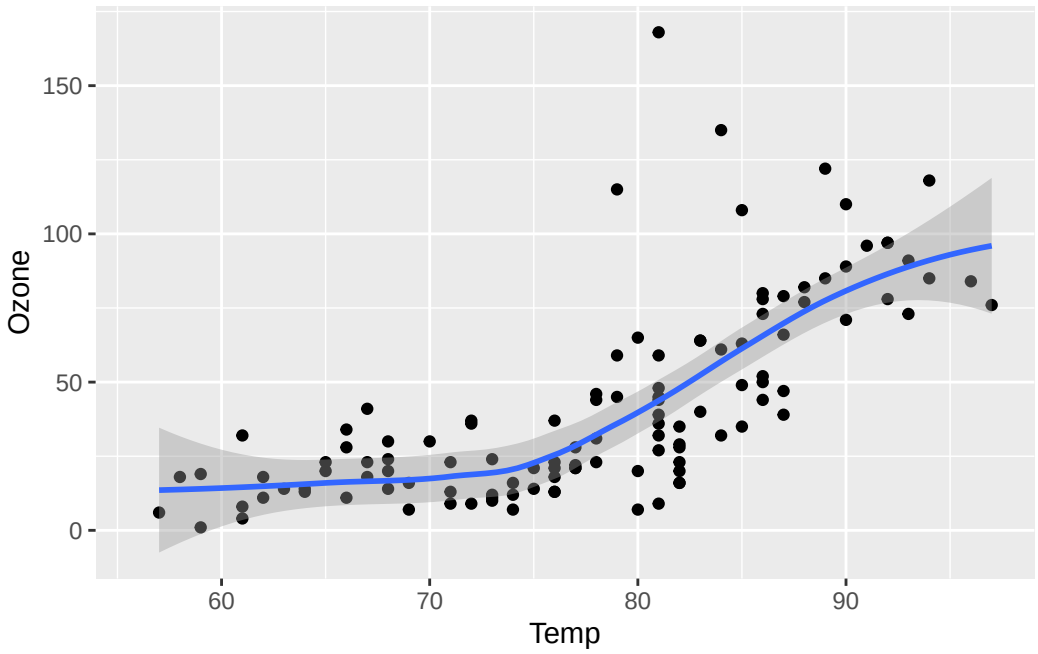

Figure 1: Temperature and ozone level.

2. ECMO circuit changes
3. Heparin Prescription 3a. cumulative dose of heparin 3b. heparin prescription changes
4. Blood products consumption

1.2 Calculated variables

1. Time in Therapeutic Range (Rosendaal Method)
2. Variability in Anticoagulation (Fihn's method)

1.3 Independent Variables

1. Demographics (age, sex, ethnicity, height, weight, BMI etc)
2. Blood variables (Full blood count results, renal function, CRP , etc)
3. Anticoagulation related variables (all the APTTr and anti-Xa results)

1.4 Time related variables

- time to any bleeding or thrombotic event
- time to only bleeding events
- time to only thrombotic event
- time to ECMO circuit change

2 DATA SOURCE

- Electronic health records data extraction from GSTT systems using ECMO pump speed as definition of cases.
- Cases cross-checked with ECMO VV referral system database.
- Complications individually reviewed where relevant.
- Electronic health records accessed through GDPR compliant system and saved in encrypted work spaces.
- All investigation results (CT imagings, blood results) in between ECMO run times are extracted.
- All prescription related data (e.g., heparin infusions are extracted to the nearest hour)

3 GROUND TRUTHS

3.1 3.1. Assumptions

3.1.1 Assumption 1 : each patients receive only 1 ECMO run

- verified through discussion with clinicians at regular mortality and morbidity meeting

3.1.2 Assumption 2: all ECMO pump speed run times are accurate with automatic entry to EHR system

- verified through daily clinical use
- Thus, this time period is used as a basis to calculate other times in relation.

3.1.3 Assumption 3: As mandated legally by NHS Blood and Transfusion, all blood products transfusion are documented.

- This was used to verify and cross-check ECMO run times and related complications.
- all blood products prescribed and given are checked and confirmed if there are discrepancies.
- Assumed that the blood product is given in whole – e.g., if 1 unit Packed Red blood cells are prescribed, it is assumed the whole unit is given.

3.2 3.2. Definitions

3.2.1 Outcome variables

3.2.2 Bleeding and Thrombotic Complications

Any relevant bleeding and thrombotic complications simultaneously occuring and documented in medical notes are recorded and nearest most accurate time period extracted.

This does not include complications sustained through ECMO cannulation process nor incurred through retrieval process.

In addition, relevant complications identified incidentally through cross sectional imaging are also supplemented.

EOLIA definition of bleeding complications restrict to bleeding events requiring transfusion of blood products.

Our definition of bleeding complication is more broad. This is to allow analysis at granular scale.

We separately analyse blood products transfusion as a proxy marker of complications.

3.2.3 Time to events

Our analysis is to answer the question of the impact of monitoring tool after being established on anticoagulation for VV ECMO.

As a result, our time to “first” BTE is defined as the first BTE after having established on VV ECMO. Thus, the complications at admission associated to pre-ECMO status or associated to cannulation is not counted. The time starts at the time of successful cannulation and arrival to GSTT ECMO unit and ends at the time of first BTE.

Similarly, the ECMO circuit change is counted as the first circuit change after being established on VV ECMO.

3.2.4 ECMO circuit changes

Similarly, documentation of ECMO circuit changes and rationales were extracted.

3.2.5 Heparin administrations

All administration of heparin prescribed under different regimes were extracted and relevant units obtained.

Regimes	Description	Units
1	Heparin	units per hour
2	Heparin ECMO Xa IV Infusion	units per kilogram per hour
3	Heparin IV injection	units
4	Heparin RR INF 20000 IU	units
5	Heparin systemic Inf 20000 Units	units per hour

3.2.6 Independent Variables

3.2.7 Height and Weight

Original documentaion of height and weight on ECMO cannulation were used due to likely later variation of patients through out their critical illness.

3.2.8 Time in Therapeutic Range (Rosendaal method)

Linear interpolation method by Rosendaal was used, using the following formula. Each individual time period between each anticoagulation blood test was calculated to the nearest hour. Then Time in Range for that period was calculated. Finally, for each patient and their ECMO duration, the cumulative TTR was calculated.

$$Time\,in\,Therapeutic\,Range\,_{Rosendaal} = \frac{Time\,in\,range}{Total\,Time}$$

No of anticoagulation blood tests per day was calculated to detect anomalous results. When outlier values were noted and duplicate record with non outlier value was found, this was chosen and randomly verified manually.

3.2.9 Variability of Anticoagulation (Fihn's method of Variance Growth rate)

$$\sigma^2 = \frac{1}{n} \sum_{i=1}^{n} \frac{(value_i - target)^2}{\tau_i}$$

Variance Growth Rate is a derivation of a statistical measure "variance" adjusted for each individual time period.

For each value of anti-coagulation test result, this was substracted from target and squared, then divided by time period. Summary of all of this for the entire duration of ECMO was then calculated to generate a final value of VGR for each patient.

3.2.10 Targets of anticogulation

For aPTTr : target range xxx is thus, target value xxx is chosen as midpoint and

For anti-Xa : target range xxx is , and mid point xxx is chosen as target value ### Blood related variables - All blood related variables are extracted and first 24 hour blood values summarised.

https://onlinelibrary.wiley.com/doi/10.1111/jth.12322 # DATA CLEANING PROCESS

All relevant variables and time-stamps are verified to be within ECMO run times.

Categorical variables are summarised and visualised. Numeric variables are summarised and visualised to allow for visual inspection of distribtion (e.g., normal distribution or kurtosis).

If data is transformed (e.g., log transformation), post transformation distribution is visualised

Count data is visualised and modeled as poisson / quasi-poisson distribution.

4 DESCRIPTIVE ANALYSIS

Supplementary Table 1 of the first 24-hour admission blood results variables

Blood investigation variables	aPTTr group	anti-Xa group	p Value
	(N=74)	(N=180)	
Haemoglobin (g/L)			
Mean (SD)	101 (15.2)	99.8 (14.8)	0.656
Median [Min, Max]	99.8 [71.0, 139]	98.0 [70.5, 147]	

Blood investigation variables	aPTTr group	anti-Xa group	p Value
Platelets (10 ^9/L)			
Mean (SD)	152 (94.9)	231 (102)	<0.001
Median [Min, Max]	141 [17.0, 390]	219 [0, 495]	
Neutrophils (10 ^9/L)			
Mean (SD)	9.26 (7.29)	12.0 (5.44)	<0.001
Median [Min, Max]	7.48 [0.100, 41.9]	11.0 [0.950, 31.9]	
Fibrinogen (g/L)			
Mean (SD)	4.89 (2.16)	5.49 (2.49)	0.086
Median [Min, Max]	4.70 [0, 9.70]	5.38 [0.500, 11.8]	
Lactate dehydrogenase (U/L)			
Mean (SD)	616 (405)	793 (1130)	0.389
Median [Min, Max]	528 [0, 2390]	558 [0, 9840]	
Ferritin (ug/L)			
Mean (SD)	2200 (5150)	2760 (5320)	<0.001
Median [Min, Max]	70.0 [0, 36200]	1330 [0, 51500]	
Creatinine Kinase (umol/L)			
Mean (SD)	2200 (4380)	2640 (23600)	0.00771
Median [Min, Max]	620 [0, 22600]	327 [0, 317000]	
C-reactive protein (mg/L)			
Mean (SD)	223 (106)	178 (147)	0.00292
Median [Min, Max]	213 [53.5, 515]	151 [0, 622]	
Procalcitonin (ug/L)			
Mean (SD)	16.2 (29.1)	6.90 (15.9)	0.033

Blood investigation variables	aPTTr group	anti-Xa group	p Value
Median [Min, Max]	2.79 [0, 100]	0.913 [0, 91.6]	
Bilirubin (umol/L)			
Mean (SD)	21.3 (19.9)	17.2 (30.4)	0.0666
Median [Min, Max]	14.0 [0, 127]	11.0 [0, 389]	
Albumin (g/L)			
Mean (SD)	24.6 (3.86)	26.7 (3.99)	<0.001
Median [Min, Max]	24.0 [15.5, 35.0]	27.0 [18.0, 39.5]	
Creatinine (umol/L)			
Mean (SD)	170 (117)	120 (106)	<0.001
Median [Min, Max]	146 [34.0, 671]	75.3 [21.0, 626]	
eGFR (needed)			
Mean (SD)	58.0 (45.6)	89.8 (55.2)	<0.001
Median [Min, Max]	38.8 [6.00, 208]	91.3 [0, 330]	
Corrected Calcium (umol/L)			
Mean (SD)	2.34 (0.147)	2.30 (0.283)	0.0851
Median [Min, Max]	2.36 [1.83, 2.64]	2.32 [0, 3.01]	
Bicarbonate (mmol/L)			
Mean (SD)	24.9 (5.51)	28.1 (3.97)	<0.001
Median [Min, Max]	23.4 [10.7, 38.6]	28.2 [15.0, 37.6]	
Lactate (mmol/L)			
Mean (SD)	3.10 (3.34)	1.85 (1.39)	0.342
Median [Min, Max]	1.60 [0.700, 18.2]	1.60 [0.600, 16.8]	
pH			
Mean (SD)	7.37 (0.0604)	7.36 (0.553)	<0.001
Median [Min, Max]	7.37 [7.06, 7.46]	7.40 [0, 7.47]	

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6 8. Models

6.1 8.1. Model 1 : Time-in-Therapeutic-Range (TTR)

6.1.1 Model used: 5-part1-analysis.R

6.1.2 bmxphi

6.1.3 Interpretation

Membership of anti-Xa group decreases by 0.95 the odds of having a larger TTR. Each additional day on ECMO increases the odds of having a larger TTR by 1.01. Receipt of renal replacement therapy decreases the odds of having a large TTR by 0.6.

6.2 https://journals.sagepub.com/doi/10.1177/0962280217690413?url_ver=Z39.88-2003&rfr_id=ori%3Arid%3Acrossref.org&rfr_dat=cr_pub++0pubmed

for interpretation

6.2.1 Beta regression assumptions

Time in Therapeutic Range is an intuitive index of measure for measuring quality of anticoagulation.

Several definitions of TTR is available :-
- Traditional - Linear interpolation method by Rosendaal et al

Traditional = no of tests in range divided by total no of tests. This does not take into account duration. Thus, Rosendaal et al has calculated a linear interpolation method incorporating duration of measurements.

Here, TTR(Rosendaal) is used.

TTR is a value of range between 0 to 100%. Thus, beta-regression is used, using {betareg} package.

TTR includes values including 0 and 100%, whereas strictly beta-regression does not include values 0 or 100%. Thus, data-transformation y1 = y * n-1) + 0.5 / n as per Smithson and Vekuilen was carried out.

Above findings confirmed that data transformation was appropriate.

It was hypothesised that 1. age 2. BMI 3. sex 4. apache II score 5. monitoring group 6. renal replacement therapy 7. duration on ecmo 8. admission period median pH value

are likely to affect “time-in-therapeutic range”

6.2.2 Multi-variate model fitting

Beta regression model using above 8 variables were fitted. Step-wise variable selection was undertaken using Akaike Information Criteria. Likelihood ratio test {lmtest} using function “lrtest” was used to evaluate final model against a null model.

Likelihood ratio test confirms that p-value of final model is 0.000171 compared to null model

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In this model, variables “sex, renal replacement therapy, and duration of ECMO” are the only variables that have statistical significant. Thus, a reduced model using only this 3 variables were fitted.

Reduced model and full models were compared using likelihood ratio test and there were not statistically significant differences.

Reduced model AIC was lower than full model AIC by 5 points. As a result, we have selected a full variable model for its ability to infer effects of biologically plausible variables such as age, sex.

APACHE 2 score already includes pH value and APACHE 2 score was not known to be predictive of outcome in ECMO patients. Thus, sensitivity analysis was undertaken with both variables - APACHE 2 score and median pH value. Models were evaluated using AIC and likelihood ratio test. Likelihood ratio tests found that model including only APACHE score without pH variable has the lowest chi-square value and is statistically significant ($p<0.0001$).

Thus, this model was evaluted further.

6.2.3 Variable Dispersion

In the final model, dispersion parameter - phi coefficient- was estimated at 2.27 and was statistically significant. The most likely variable contributing to dispersion was ‘duration of ecmo’.

Thus, final model was re-fitted with the same mean equation but now with duration of ECMO as additional regressor for the precision parameter - phi.

https://cran.r-project.org/web/packages/betareg/vignettes/betareg.pdf

The model including of ecmo duration as regressor for the precision parameter was statistically significant and improved a model fit, without significant difference in estimates of other paramters. AIC of this new model was significantly lower than model without precision parameter.

Thus, there was a statistically significant evidence for variable dispersion, and thus was chosen as a final model

6.2.4 Fit assessment

Maximum likelihood estimation was used to calculate p-values.

Model assumptions were also evaluated using diagnostic plots; and was graphically satisfacotry for normal assumption, homeoskedasticity, and influential observations effects.

Heteroskedasticity was also checked numerically using studentized Bresuch-Pagan test and demonstrated no evidence of heteroskedasticity.

Multicollinearity was assessed using variable inflation factors using {car} package function “vif”. All variables have VIF score < 2 demonstrating no evidence of multi collinearity.

Link function of logit is used.

ttrpl4

6.3 8.2. Model 2 : Variability of Anticoagulation

6.4 Model chosen : mox02

6.5 Interpretation :

for 1 unit increase in lactate, our variability increases by 10.6% membership in anti Xa decreases variability by 86%. Variability of anticoagulation is a measure of quality of anticoagulation. Fihn’s method of variability was used for this study.

Lower Variability results in better control of anticoagulation

6.5.1 Choice of model and assumptions

Variability of anticoagulation was significantly right skewed thus, a natural logarithmic transformation was undertaken of dependent variable and then a linear model was fitted against.

It was hypothesised that 1. age 2. BMI 3. sex 4. apache II score 5. monitoring group 6. renal replacement therapy 7. duration on ecmo

were thought to be affecting variability of anticoagulation.

6.5.2 Multi-variate modelling

On a multi-variate modelling, as evaluated by AIC and likelihood ratio tests, monitoring group and lactate are the only two statistically significant variables.

Thus,a reduced model using only statistically significant model was evaluated against a full model - there were no improvement of a reduced model. And, due to ability to infer effects of other biologically plausible variables, age, bmi, apache etc are included in a final model.

6.5.3 Fit assessment

Multiple R squared value of fitted model was 0.5 and model was statistically significant.

Model assumptions were checked for normality, heteroskedasticity, effect of outlying values and distribution of residuals.

Numerical check of final model using Breusch-Godfrey test confirmed visual findings that model residuals are homoskedastic.

6.5.4 Reporting

https://data.library.virginia.edu/interpreting-log-transformations-in-a-linear-model/

6.6 8.3.

6.7 8.4.

6.8 8.5.

6.9 8.6.

6.10 8.7. Model 7 : Cumulative Dose of Heparin

6.10.1 Model Choice

- log transformed so interpret as per above

6.10.2 Multi-variate analysis

interaction was checked ttrg:group AIC lrtest

6.10.3 Fit assessment

6.10.4 Reporting

7 9. Summary

8 To Do

1. when reading the manuscript, please consider if we fulfill STROBE guidelines
2. please complete the “units” for measured variables in table-1(e.g., CRP, creatinine)
3. please vote on which figures to include
4. two-sentence take home message and 140-character tweet
5. 250-word abstract and 3-5 keywords
6. Agree on title
7. Suggest list of reviewers – Nunez, Schmidt, Alain V?

https://www.springer.com/journal/134/submission-guidelines?detailsPage=pltci_1060748#Instructions%20for%20Authors_Authorship%20principles