Supplementary Material : Accuracy and Precision of anticoaguation tests and impact on bleeding and thrombotic events during ECMP

Teddy Tun Win HLA on behalf of the study group

Table of contents

1 ANTICOAGULATION PROTOCOL: Unfractionated Heparin Infusions for Critically Ill Adult Patients - anticoagulation and anti-Xa testing.

An abbreviated summary of GSTT's anticoagulation protocol is described here. For full clinical guideline, please contact the authors.

The guideline was developed by Dr Andrew Retter, Lynda Cameron and Fraser Hanks and approved by Thrombosis and Thromboprophylaxis committee. The guideline is routinely reviewed yearly for update and amendments.

This guideline focuses on the use of systemic intravenous unfractionated heparin infusions (UFH) for the anticoagulation of patients in Adult Critical Care who are receiving Mechanical Circulatory Support (MCS) – including intra-aortic balloon pumps (IABP), Impella devices, VV-ECMO, VA-ECMO and VVAECMO. It excludes patients in whom treatment for venous thromboembolism (VTE) is intended. This guideline is for use at Guy's and St. Thomas' sites only.

1.1 Initiation

Baseline monitoring prior to commencing a UFH infusion is done with coagulation screen and full blood count. The initial UFH infusion rate is started at 18 units per kilogram per hour using adjusted body weight (or total body weight if total body weight is less than adjusted weight). No initial loading dose or bolus is to be administered in critically ill patients.

1.2 Monitoring and Titration

Anti-Xa level are checked four hours after the initiation of the UFH infusion and /or 4 hours after any rate change.

Once two consecutive, anti-Xa levels are within thereapeutic range, without a pause/interruption to the infusion or a dose adjustment being required, anti-Xa monitoring can be reduced to once a day. At this point, anti-Xa level should be sent with routine monitoring morning bloods.

1.3 Caveats in Critical illness

In critical illness, there is often a discrepancy between aPTTr and anti-Xa levels. Please measure aPTTr every 2nd day, anti-Xa level is usually consistent with aPTTr between an anti-Xa range of 0.3 to 0.7.

If the APTTr is more than 3.0, regardless of the anti-Xa level, inform the critical care consultant and seek Thrombosis team advice (consultant on call or specialist bleep 0122).Please measure an antithrombin activity level and repeat the anti-Xa and APTTr levels, ensuring sampling from a non-heparin contaminiated line

1.4 Troubleshooting : identification of heparin resistance anti-thrombin deficiency

- If the patient is receiving more than 2000 units/ hr of heparin, please send an antithrombin activity.
- If two consecutive anti-Xa levels are subtherapeutic, despite increasing heparin rate, please send an anti-thrombin activity level.
- if the anti-thrombin level is less than 50 international units, please discuss with the critical care consultant.
- If the patient develops a thrombus whilst on heparin, review with consultant and consider switching to an argatroban infusion
- An anti-Xa level more than 1.0units/mL is VERY HIGH, with a concordant risk of spontaneous haemorrhage. The infusion should be paused, consultant informed, and the level should be urgently rechecked. Take care to ensure that the sample is not being taken from a heparin contaminated line. Whilst waiting for the repeat result, the infusion must remain STOPPED.
- If the patient has an anti-Xa level within the target range but an APTTr greater than 3.0, please discuss with the thrombosis team. A discrepancy of this size suggests a more severe derangement of the patient's coagulation secondary to either a consumptive coagulopathy, liver disease or a lupus anticoagulant. Rarely, a large discrepancy between the APTTr and anti-Xa may suggest heparin induced thrombocytopaenia
- Wherever possible, no procedures should be undertaken until the anti-Xa level is less than 0.3units/mL. The heparin infusion should be held and the anti-Xa level checked every 4 hours until it is less than 0.3 units/mL, to allow the procedure to take place with minimal risk of bleeding complications. Post procedure, the infusion should be restarted as per the initial infusion rate. No bolus should be given.

1.5 Management in relation to VV-ECMO decannulation

- UFH infusion to be held for 4 hours prior to ECMO decannulation.
- Once decannulated, the heparin infusion to be restarted 1 to 2 hours provided there are no bleeding concerns. Restart at the same rate as prior to decannulation. Anti-Xa levels should be sent 4 hours after UFH infusion has been started.
- Doppler Ultrasound scan of both legs should be completed within 24 hours of decannulation.
- Continue the heparin infusion and anti-Xa monitoring until the results of the leg dopplers are available. If no thrombosis, switch to low molecular weight heparin prophylactic dose.
- If ongoing therapeutic anticoagulation is indicated, consider switching to therapeutic dose low molecular weight heparin if creatinine clearance is greater than 30ml/min.

2 VARIABLES

2.1 Outcome variables

1. Bleeding or thrombotic events(BTE)

1a. no BTE

1b. only haemorrhagic complications

1c. only thrombotic complications

1d. both BTE

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

2.2 Calculated variables

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

2.3 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)

2.4 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

3 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)

4 ASSUMPTIONS & DEFINITIONS

4.1 Assumptions

4.1.1 Assumption 1 : each patients receive only 1 ECMO run

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

4.1.2 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.

4.1.3 Assumption 3: All blood products transfusion are documented.

- Mandated by NHS Blood transfusion all blood product transfusions are explicitly 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.

4.2 Definitions

4.2.1 Outcome variables

4.2.1.1 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.

4.2.1.2 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.

4.2.1.3 ECMO circuit changes

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

4.2.1.4 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

Table of heparin administration types.

4.2.2 Independent Variables

4.2.2.1 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.

4.2.2.2 Time in Therapeutic Range (Rosendaal method)

Time in Therapeutic Range is an intuitive measure for evaluating the quality of anticoagulation. Multiple definitions exist.

Traditional definition is effectively no of tests in range as numerator divided by total no of tests. This does not take into account different durations that patient are anticoagulated for. Thus, Rosendaal et al has calculated a linear interpolation method incorporating duration of measurements.

TTR is a value of range between 0 to 100%.

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.[9]

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

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.

4.2.2.3 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.[1]

For each value of anti-coagulation test result, this was subtracted 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.

The benefit of Variance Growth Rate is that this does not assume the patient to be in a target range(which TTR does). VGR calculates how "far" each value is from target range adjusted for time period.

4.2.3 Targets of anticogulation

For aPTTr : target range 1.5 to 2.0 is thus, target value 1.75 is chosen as midpoint and

For anti-Xa : target range 0.3 to 1.0 is , and mid point 0.65 is chosen as target value

4.2.4 Blood related variables

- All blood related variables are extracted and first 24 hour blood values summarised.

5 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.

Correlation between numerical values are visualised with correlograms and pearson coefficients calculated.

6 DESCRIPTIVE ANALYSIS

Supplementary Table 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]	
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

Blood investigation variables	aPTTr group	anti-Xa group	p Value
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
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]	

Blood investigation variables	aPTTr group	anti-Xa group	p Value
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]	

7 MULTIVARIATE MODELS

7.1 Outcome 1a : Time-in-Therapeutic-Range (TTR)

Table 3: TTR multivariable analysis

	ttrgf		
Predictors	Estimates	95% CI	p Value
(Intercept)	0.56	0.20 – 1.54	0.26
age	1.01	1.00 – 1.03	0.14
sex [M]	0.78	0.59 – 1.05	0.10
bmi	1.01	0.99 – 1.03	0.28
apache	0.99	0.95 – 1.03	0.63
group [gaxa]	0.95	0.68 – 1.32	0.74
rrt [yes]	0.60	0.43 – 0.85	**<0.01**
ecmod	1.01	1.00 – 1.02	**0.01**
Observations	254		
R^2	0.087		

7.1.1 Model : multi-variate beta regression (family of generalised linear models)

7.1.2 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.

7.1.3 Beta regression assumptions

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 / as per Smithson and Vekuilen was carried out.[10]

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". Beta regression model using above 8 variables were fitted. Variable selected and their impact on final model was reviewed 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.

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 selected further.

7.1.4 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.

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.

7.1.5 Fit assessment

Maximum likelihood estimation was used to calculate p-values.

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

Heteroskedasticity was also checked numerically using studentized Bresuch-Pagan test and demonstrated no evidence of heteroskedasticity. Heteroskedasticity is usually well-managed by betaregression given its ability to handle extremes of data with phi coefficient.

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.

Reporting of effect size was done by exponentiating the model coefficients and reported as 'odds per units of x_i as per[2] [8].

7.2 Outcome 1b : Variability of Anticoagulation as VGR

Table 4: Multivariable analysis of VGR

	sigm 2		
Predictors	Estimates	95% CI	p Value
(Intercept)	-0.61	-1.99 – 0.77	0.39
age	0.00	-0.01 – 0.01	0.98
bmi	0.01	-0.01 – 0.03	0.40
apache	0.01	-0.03 – 0.05	0.64
sex [M]	-0.02	-0.31 – 0.27	0.89
group [gaxa]	-2.04	-2.38 – -1.70	**<0.01**
alb median	0.01	-0.03 – 0.05	0.54
lactate median	0.11	0.04 – 0.18	**<0.01**
plt median	0.00	-0.00 – 0.00	0.29
rrt [yes]	0.22	-0.14 – 0.58	0.23
ecmod	-0.00	-0.01 – 0.01	0.68
Observations	253		

	sigm 2
R^2 / R^2 adjusted	0.504 / 0.484

7.2.1 Model : Multivariable linear regression with outcome log transformed and interpreted accordingly

7.2.2 Interpretation

Variability of anticoagulation is a measure of quality of anticoagulation, with lower Variability results in better control of anticoagulation. For 1 unit increase in lactate, our variability increases by 10.6% membership in anti Xa decreases variability by 86%.[4]

7.2.3 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.

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.

7.2.4 Fit assessment

Multiple R squared value of fitted model was 0.5 and model was statistically significant, whilst R squared value is not encouraging, it offers a bility to infer the reduction in variability in anti-Xa group whilst adjusting for other varibles.

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.

7.3 Outcome 2a : Rate of bleeding and thrombotic events

7.3.1 Model : Multivariate Poisson distribution using ecmo duration as offset

Dependent variable is selected for “any” bleeding and thrombotic events. Each event is considered as seperate event, thus discrete count.

Table 5: Rate of any bleeding or thrombotic events

	abte		
Predictors	Incidence Rate Ratios	95% CI	p Value
(Intercept)	0.17	0.06 – 0.46	**<0.01**
age	1.01	0.99 – 1.02	0.25
sex [M]	0.72	0.55 – 0.95	**0.02**
bmi	0.99	0.97 – 1.01	0.37
apache	0.98	0.94 – 1.02	0.30
group [gaxa]	1.07	0.77 – 1.50	0.70
ttrg	0.16	0.10 – 0.25	**<0.01**
sigm	0.99	0.96 – 1.01	0.55
rrt [yes]	1.37	0.99 – 1.88	0.05
Observations	254		
R^2 Nagelkerke	0.327		

Given the count nature of “events” data, a dependent variable is visualised and found to conform to poisson distribution. Thus, this is modeled as multivariable poisson regression using a standard “log” link function.

However, it is important to note that there are statistically significant differences and variation of ECMO duration between two different study groups. The longer duration on ECMO, the longer the duration of anticoagulation and the potential risk of any complication is higher.

This is taken into account by using an “offset” of ecmo duration.

As per above, model assumptions checked.

7.4 Outcome 2b : Rate of only bleeding events

7.4.1 Model : Multivariate Poisson distribution using ecmo duration as offset

Table 6: Only haemorrhagic complication events

	toth		
Predictors	Incidence Rate Ratios	95% CI	p Value
(Intercept)	0.02	0.00 – 0.11	**<0.01**
age	1.03	1.00 – 1.05	**0.02**
sex [M]	0.87	0.56 – 1.37	0.53
bmi	1.01	0.98 – 1.04	0.48
apache	1.00	0.94 – 1.06	0.90
group [gaxa]	0.64	0.37 – 1.12	0.12
ttrg	0.12	0.06 – 0.25	**<0.01**
sigm	0.86	0.70 – 0.99	0.10
rrt [yes]	1.51	0.93 – 2.44	0.09
Observations	254		
R^2 Nagelkerke	0.276		

As per above but restricted to analysis of only “haemorrhagic complications”. Similar treatment was followed.

7.5 Outcome 2c : Rate of only thrombotic events

7.5.1 Model : Multivariate Poisson distribution using ecmo duration as offset

Table 7: Thrombotic Complications only

	totthr		
Predictors	Incidence Rate Ratios	95% CI	p Value
(Intercept)	0.28	0.07 – 1.11	0.07
age	1.00	0.98 – 1.02	0.85
sex [M]	0.57	0.39 – 0.83	**<0.01**
bmi	0.98	0.95 – 1.00	0.06
apache	0.95	0.90 – 1.00	0.07
group [gaxa]	1.57	0.96 – 2.64	0.08
ttrg	0.22	0.12 – 0.43	**<0.01**
sigm	1.00	0.98 – 1.01	0.82

	totthr		
rrt [yes]	1.34	0.85 – 2.09	0.21
Observations	254		
R^2 Nagelkerke	0.180		

As per above, but for only thrombotic complications.

7.6 Outcome 2d : Rate of ECMO circuit change

7.6.1 Model : Multivariate Poisson distribution using ecmo duration as offset

Table 8: ECMO circuit change analysis

	totc		
Predictors	Incidence Rate Ratios	95% CI	p Value
(Intercept)	0.04	0.01 – 0.12	**<0.01**
age	1.01	1.00 – 1.02	0.14
sex [M]	1.19	0.89 – 1.60	0.25
bmi	1.01	1.00 – 1.03	0.15
apache	0.97	0.93 – 1.01	0.17
group [gaxa]	0.68	0.48 – 0.95	**0.02**
ttrg	0.92	0.56 – 1.51	0.73
sigm	0.97	0.90 – 1.01	0.32
rrt [yes]	1.30	0.94 – 1.79	0.10
Observations	254		
R^2 Nagelkerke	0.108		

The next or first ECMO circuit change after cannulation on VV ECMO first are modelled against independent variables.

The ECMO circuit change events are modelled as count data - poisson regression- is used again using ecmo duration as an offset.

7.7 Outcome 2e : Cumulative Dose of Heparin

7.7.1 Model : Multivariate linear regression with log transformed outcome and interpreted accordingly

Table 9: Cumulative dose of heparin - log transformed

	cud 2		
Predictors	Estimates	95% CI	p Value
(Intercept)	11.38	10.78 – 11.97	**<0.01**
age	-0.00	-0.01 – 0.01	0.71
sex [M]	0.13	-0.04 – 0.29	0.12
bmi	0.01	-0.00 – 0.02	0.12
group [gaxa]	0.10	-0.15 – 0.35	0.44
lactate median	-0.13	-0.17 – -0.09	**<0.01**
ecmod	0.04	0.04 – 0.05	**<0.01**
ttrg	0.78	0.43 – 1.13	**<0.01**
sigm [log]	0.03	-0.05 – 0.10	0.52
Observations	232		
R^2 / R^2 adjusted	0.689 / 0.678		

7.7.2 Model Choice

- log transformed of heparin dose given a significantly skewed distribution of heparin.
- Given the likely interaction of duration of ecmo, interaction between ecmo duration and TTR & ecmo duration and VGR are included in an extended model.
- The model fit is not improved and the interactions are not statistically significant.
- Thus, the reduced model without interaction effects are chosen.

7.7.3 Fit assessment

- Linear regression fit assessment as per AIC, and likelihood ratio tests.

7.7.4 Interpretation

Given log transformation of dependent variable, the effect size is interpreted as a percentage change.

7.8 Outcome 2f : Rate of change of heparin prescriotion

7.8.1 Model : Multivariate Poisson distribution using ecmo duration as offset

Table 10: Analysis of heparin prescription changes

	runl		
Predictors	Incidence Rate Ratios	95% CI	p Value
(Intercept)	0.72	0.37 – 1.35	0.31
age	1.00	0.99 – 1.00	0.25
sex [M]	1.01	0.87 – 1.17	0.93
bmi	1.00	0.99 – 1.01	0.80
group [gaxa]	1.37	0.91 – 2.10	0.14
lactate median	0.94	0.89 – 1.00	**0.04**
ttrg	3.11	1.74 – 5.74	**<0.01**
sigm	1.01	1.00 – 1.01	**0.01**
rrt [yes]	1.12	0.94 – 1.33	0.19
group [gaxa] × ttrg	0.28	0.14 – 0.53	**<0.01**
Observations	254		
R^2 Nagelkerke	0.665		

Rate of change of heparin prescription is modelled as count data, poisson generalised model, with an offset for ecmo duration.

The interaction between TTR and monitoring is found. It is stastically significant with p<0.001 and thus included.

7.9 Outcome 2g : Rate of blood products transfused per ECMO duration

7.9.1 Model : Multivariate Poisson distribution using ecmo duration as offset

Our definition of BTE is more relaxed than ELSO definition since we count all documented and imaged BTEs. The ELSO definition of bleeding event is defined as that requiring blood product transfusion.

Given the predominance of red blood cell transfusion over other blood products and given we are looking at overall effect on coagulation, we have modelled all blood together summatively.

Table 11: Total blood products transfused

	bldtot		
Predictors	Incidence Rate Ratios	95% CI	p Value
(Intercept)	0.36	0.22 – 0.59	**<0.01**
age	1.00	1.00 – 1.01	0.08
sex [M]	1.05	0.94 – 1.18	0.37
bmi	0.99	0.99 – 1.00	**0.04**
apache	1.05	1.03 – 1.06	**<0.01**
rrt [yes]	1.61	1.42 – 1.83	**<0.01**
cudose	1.00	1.00 – 1.00	**0.03**
ttrg	0.24	0.16 – 0.36	**<0.01**
sigm [log]	1.07	1.02 – 1.12	**<0.01**
group [gaxa]	0.78	0.60 – 1.01	0.05
ttrg × group [gaxa]	1.57	1.01 – 2.44	**0.05**
Observations	232		
R^2 Nagelkerke	0.943		

Blood products consumption were modelled as a count data using multivariate poisson regression.

The interaction were found between time in therapeutic range and monitoring group and was statistically significant.

7.10 Outcome 2h : Time to any bleeding or thrombotic complication

7.10.1 Model : Multivariate Cox proportional hazards model

Given the frequency of BTEs in any ECMO population and clinicians tendency to change "anticoagulation targets" after a BTE event (e.g., a thrombotic event may warrant a higher therapeutic target and a bleeding event may warrant a pause in anticoagulation or a lowering of anticoagulation target).

As a result, we have analysed the time to first BTE, this is because it is likely that after the first BTE, anticoagulation targets are likely to be adjusted and thus affecting on TTR and VGR by clinical team.

The BTE detected through admission CT scan are not counted as this is likely to be due to pre ECMO or cannulation related events whether anticoagulation may not have been started.

Similar rationale is discussed in the literature to delineate BTE associated with ECMO from other concurrent events such as procedures, surgery or initial cannulation period.[7]

With this assumptions, we explored time to BTE using multivariable cox proportional hazards model.

Table 12: Cox model for time to first any BTE

	Dependent variable		
Predictors	Estimates	95% CI	p Value
age	1.03	1.00 – 1.05	**0.04**
sexM	0.92	0.58 – 1.46	0.72
rrtyes	1.16	0.63 – 2.13	0.63
bmi	0.99	0.96 – 1.01	0.32
groupgaxa	0.97	0.56 – 1.67	0.90
apache	0.94	0.87 – 1.01	0.08
ttrg	0.17	0.07 – 0.41	**<0.01**
sigm	1.00	0.96 – 1.03	0.79
Observations	192		
R^2 Nagelkerke	0.098		

The duration on ECMO is one of the major factors influencing the analysis of time to “first” BTE in ECMO.

Duration on ECMO is highly variable with significant outliers with long tail distribution where some patients surviving ECMO after over 50 days. COVID pneumonitis patients in our population would also be over-represented given their higher absolute numbers and longer duration of ECMO time.

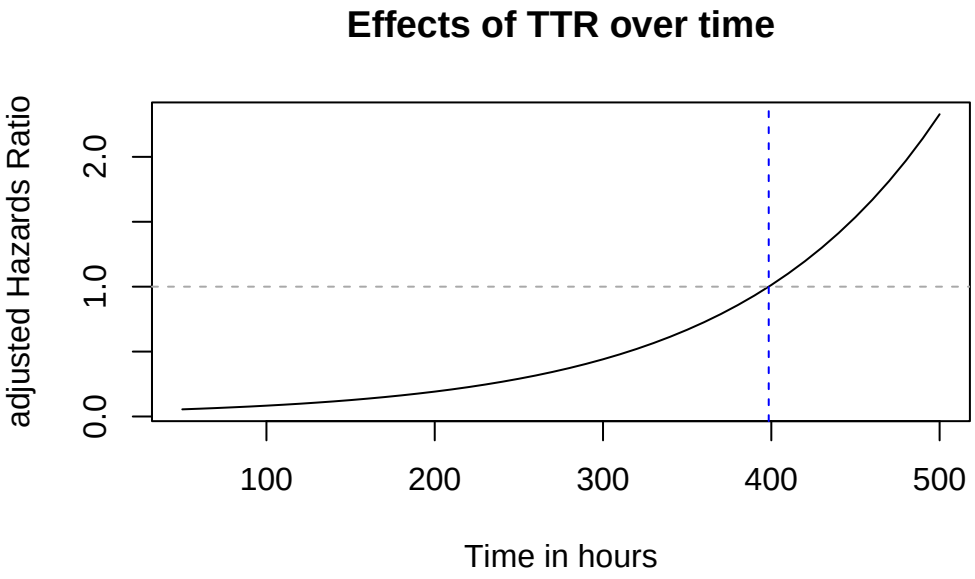
Our median time on ECMO is 11 and 17 days respectively for aPTTr and AXA groups. This is consistent with a large multi-centre international study evaluating ECMO in COVID-19 pneumonitis with over 844 ECMO patients reporting a median time on ECMO as 13 days.[11]

As a result, we have undertaken a pragmatic cut off of 20.8 days (500 hours).

This results in 192 patients who have 92 BTE events within this period.

The major findings from our model are that age is associated with higher risk of bleeding and thrombotic outcome (adjusted Hazard Ratio 1.03, p = 0.042), and higher value of time in therapeutic range is protective against having BTE in the initial period (adjusted HR 0.17, p<0.001).

However, TTR has an statistically significant (p = 0.0261)interaction with time and the initial protective effect of high TTR changes after a certain period on ECMO.



7.11 On modelling VGR

Variance Growth Rate is a summary value with ranges including 0 and is significantly skewed. The statistical methods used in modelling skewed distribution is commonly to undertake "log transformation". Hence, most of the literature that analyses VGR uses log-transformed i.e., log VGR. [3] [razouki_improving_2014] [van_miert_effect_2020].

Whilst this is a successful statistical treatment for vitamin K antagonists, where target range is usually > 1 this does not always apply to our data where our target range has values < 1.

Given the minimal impact of VGR in this study and given the ease of interpretability of non-transformed VGR, we have only performed log transformation where relevant as an acceptable trade off to overall assess the impact of VGR and TTR.

7.12 On Using Poisson models

7.12.1 Offset

Given a significant differences in median duration of ECMO between two population, it is prudent to adjust for this effect whilst analysing events ("counts")

An intuitive treatment would be a simply using ECMO duration as a denominator to the events, however, a simple division assumes linearity (e.g., if 1 circuit change or 1 transfusion of red cells in 3 days, then would expect a 2nd circuit change or 2nd transfuion by day 6)

Therefore, we have modeled using a ECMO duration as *"offset"* in Poisson models. This is a commonly accepted approach and routinely used in analysis of similar circumstances. For example, Nijman (2022) using poisson modes to calculate emergency room attendances during COVID 19 pandemic whilst using population as "offset" to adjust for case-mix differences. [6] Kanankege(2022) used a human population as offset in Poisson model of high risk areas for dog-mediated rabies.[5]

7.12.2 Comparison of poisson models with quasipoisson, and zeroinflated poisson models

Poisson models assume 'equidispersion' which in real-world data may not resemble a poisson distribution. Mean and variance of dependent variables of poisson models were manually checked and did not detect a significant over-dispersion.

However, zero-inflated poisson (ZIP) models is a common alternative along with quasipoisson models offering potentially better fit over poisson models.

All poisson models were re-fitted with both quasipoisson family of distribution and also with zero-inflated poisson models. Vuong Non-nested hypothesis test was used to compare model fit of poisson models and zero-inflated poisson models using AIC and BIC.

In our models, ZIP models did not offer any advantage of model fit over poisson models. Thus, poisson models were chosen.

8 STROBE Checklist

References

[1] Stephan D. Fihn et al. “Comparison of control and stability of oral anticoagulant therapy using acenocoumarol versus phenprocoumon”. In: *Thrombosis and Haemostasis* 90.2 (Aug. 2003), pp. 260–266. ISSN: 0340-6245. DOI: 10.1160/TH02-10-0179.

[2] Emilie A. Geissinger et al. “A case for beta regression in the natural sciences”. In: *Ecosphere* 13.2 (2022). _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1002/ecs2.3940, e3940. ISSN: 2150-8925. DOI: 10.1002/ecs2.3940. URL: https://onlinelibrary.wiley.com/doi/abs/10.1002/ecs2.3940 (visited on 03/10/2023).

[3] S. Ibrahim et al. “The clinical evaluation of International Normalized Ratio variability and control in conventional oral anticoagulant administration by use of the variance growth rate”. In: *Journal of Thrombosis and Haemostasis* 11.8 (2013). _eprint: https://onlinelibrary.wiley.com/doi/pdf/10.1111/jth.12322, pp. 1540–1546. ISSN: 1538-7836. DOI: 10.1111/jth.12322. URL: https://onlinelibrary.wiley.com/doi/abs/10.1111/jth.12322 (visited on 03/23/2022).

[4] *Interpreting Log Transformations in a Linear Model | University of Virginia Library Research Data Services + Sciences*. URL: https://data.library.virginia.edu/interpreting-log-transformations-in-a-linear-model/ (visited on 01/29/2023).

[5] Kaushi S. T. Kanankege et al. “Identifying high-risk areas for dog-mediated rabies using Bayesian spatial regression”. In: *One Health (Amsterdam, Netherlands)* 15 (Dec. 2022), p. 100411. ISSN: 2352-7714. DOI: 10.1016/j.onehlt.2022.100411.

[6] Ruud G. Nijman et al. “Presentations of children to emergency departments across Europe and the COVID-19 pandemic: A multinational observational study”. In: *PLoS medicine* 19.8 (Aug. 2022), e1003974. ISSN: 1549-1676. DOI: 10.1371/journal.pmed.1003974.

[7] Sven R. Olson et al. “Thrombosis and bleeding in extracorporeal membrane oxygenation (ECMO) without anticoagulation: a systematic review”. In: *ASAIO journal (American Society for Artificial Internal Organs : 1992)* 67.3 (Mar. 1, 2021), pp. 290–296. ISSN: 1058-2916. DOI: 10.1097/MAT.0000000000001230. URL: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8623470/ (visited on 01/29/2023).

[8] Eva Pfarrwaller et al. “Dynamics of career intentions in a medical student cohort: a four-year longitudinal study”. In: *BMC Medical Education* 23.1 (Feb. 27, 2023), p. 131. ISSN: 1472-6920. DOI: 10.1186/s12909-023-04102-w. URL: https://doi.org/10.1186/s12909-023-04102-w (visited on 03/10/2023).

[9] F. R. Rosendaal et al. “A Method to Determine the Optimal Intensity of Oral Anticoagulant Therapy”. In: *Thrombosis and Haemostasis* 69.3 (1993). Publisher: Schattauer GmbH, pp. 236–239. ISSN: 0340-6245, 2567-689X. DOI: 10.1055/s-0038-1651587. URL: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0038-1651587 (visited on 02/17/2022).

[10] Michael Smithson and Jay Verkuilen. “A better lemon squeezer? Maximum-likelihood regression with beta-distributed dependent variables”. In: *Psychological Methods* 11.1 (Mar. 2006), pp. 54–71. ISSN: 1082-989X. DOI: 10.1037/1082-989X.11.1.54.

[11] Martin Urner et al. “Venovenous extracorporeal membrane oxygenation in patients with acute covid-19 associated respiratory failure: comparative effectiveness study”. In: *BMJ* 377 (May 4, 2022). Publisher: British Medical Journal Publishing Group Section: Research, e068723. ISSN: 1756-1833. DOI: 10.1136/bmj-2021-068723. URL: https://www.bmj.com/content/377/bmj-2021-068723 (visited on 01/29/2023).