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9. APPENDICES

Appendix A: *In vitro* study methods

A.1 Media and chemicals

Minimum essential medium (MEM), Dulbecco's modified Eagle's medium (DMEM) (both with EBSS and L-glutamine) supplemented with fetal bovine serum (FCS) (10% vol/vol) and pennstrep fungizone 100X (1% vol/vol) were purchased from Bio-Whittaker, Adcock Ingram Scientific, Johannesburg, South Africa. Gentamycin sulphate ((50 mg/ml at 0.1% vol/vol), Phenix, South Africa), insulin ((I2767) 50 mU/L), glucagon ((G664) 16 µg/L), dexamethasone ((D4902) 67 µg/L) and epidermal growth factor (EGF (E9644) 20µg/L); from Sigma-Aldrich, Johannesburg, South Africa, were added to all media. Filter-sterilized collagenase type IV (Sigma (C5138)) was dissolved in MEM (0.68 g/425 ml) with 0.65 g $CaCl_2$, 25 ml of FCS and antibiotics as above. Collagenase solution was prepared fresh on the day of the isolation procedure. Washing buffer at 10-15^oC contained deionised water with 5% FCS, dexamethasone as above, 7.01 g/L NaCl, 0.46 g/L KCl, 0.10 g/L Ca Cl_2 and 2.383 g HEPES. Perfusion buffer at 37 ^oC contained 5% FCS, dexamethasone, 9 g/L NaCl, 0.42 g/L KCl, 2.1 g/L $NaHCO_3$, 0.9 g/L D-Glucose and 4.77 g/L HEPES. For chelation, EDTA (0.58 g/L) was added to Perfusion buffer on the day of the procedure. All buffers were oxygenated with a carbogen gas mixture (5% CO_2 and 90%O_2) during the isolation procedure. Percoll (Sigma (P16440)) was mixed 9:1 with 10X Hank's balanced salt solution (HBSS) (80g/L NaCl, 4g/L KCl, 1/Lg $MgSO_4$, 0.6g/L KH_2PO_4, 0.4g/L Na_2HPO_4 and 10g of Glucose). Solutions used for perfusing the liver during hepatectomy and transport were, firstly, 1L of clinical saline at 5-10^oC, supplemented with 0.58g EDTA, 40mU Insulin, 67 µg/L Dexamethasone and antibiotics, followed by 1L of University of Wisconsin (UW) solution at 5-10 ^oC, with insulin, dexamethasone and antibiotics. The pH of all solutions was adjusted in a sterile manner to between 7.35 and 7.4 using concentrated HCl or NaOH. insulin, glucagon, dexamethasone and EGF were added to media on the day of use.

A.2 Hepatocyte culturing, cell evaluation methods and statistics

The cell suspension received from the Centrifuge and BRAT procedures were evaluated for viability and cell count using the Trypan Blue exclusion test in a Neubauer bright-line hemacytometer. The bowl volume employed in any BRAT procedure was randomly selected. To determine the effect of oxygenating the cells and media during the BRAT procedure, in three pairs of experiments flow cytometry was conducted on hepatocytes received after oxygenation had or had not occurred. After all procedures, media aliquots were tested for pathogens in order to determine the sterility of the procedures. In addition, after both centrifuge and BRAT procedures, aliquots containing 3.5-4 x 10^6 cells were taken for seeding in 75 cm^2 cell culture flasks (Corning, Adcock Ingram, Johannesburg, SA) and subsequent culturing in a humidified CO_2 incubator at 37 degrees Celsius. Culture medium was changed 12 hours after seeding, and every 24 hours thereafter for 7 days. In order to determine the impact on hepatocyte cell cycle, flow cytometry of cells scraped from the cell culture flasks was performed 3 and 7 days after seeding.

Media samples were taken daily prior to changing the medium, to evaluate hepatocyte viability by means of LD and AST leakage and to examine the state of Cellular oxidation and Aerobic metabolism by means of the lactate to pyruvate ratio. Lactate and pyruvate concentrations were measured enzymatically, lactate at 520 nm and pyruvate at 340 nm on the Beckman Synchron LX system (Beckman kit 445875 and Sigma kit 726 respectively). The liver enzymes, lactate dehydrogenase (LD) and aspartate aminotransferase (AST) were also detected enzymatically at 340 nm using the Synchron LX system (Beckman Coulter kits 442655, 442665 respectively).

To allow the cells time to recover after the isolation procedure galactose elimination at Day 2 and urea production at Day 3 were investigated (results not presented in this study). On day 4 after isolation, cytochrome P450 activity was investigated by means of lidocaine clearance, by adding 500 µg/ml lidocaine and sampling once every hour for 3 hours. To measure lidocaine, an aliquot of the sample was spiked with bupivacaine as the internal standard. The proteins were precipitated with 1 M perchloric acid. After centrifugation, the supernatant was decanted and neutralized with 1M NaOH. Two extractions with dichloromethane followed and then the organic layers were combined and dried under a stream of dry nitrogen. The residue was redissolved in dichloromethane and analyzed by gas chromatography mass spectrometry. On completion of the lidocaine study, serum-free MEM replaced that in the flasks, and albumin production was investigated with sampling 24 hours later. Albumin concentration was determined colorimetrically by measuring at 600 nm on a Technikon RA-XT system (Miles Technikon method SM4-0131E94).

The Flow cytometry procedure involved the incubation of 1 ml of propidium iodide solution (Coulter DNA-Prep Reagents Kit) with 100 µL of a 2 x 10^6 cells/ml suspension, in the dark, for 30 minutes at room temperature. The samples were analyzed using a Beckman-Coulter Altra Flow Cytometer. A comparison of forward and side scatter data was used to gate the viable cells, in order to exclude debris from the population of cells present in the samples, while the DNA histograms indicated the relative cell cycle status of the suspension, that is, the proportion of DNA in the G0/G1, S or G2M phases. In order to examine if the cultured hepatocyte populations were proliferating normally and to determine if the isolation procedures had been sterile, daily examination of the culture flasks was performed using an Olympus CKX41 inverted microscope set for phase contrast. Digital micrographs, using an Olympus C4040 camera, were taken at day 3 and day 7 after the seeding of the culture flasks.

Statistical Analyses

GraphPad Prism 2.01 was used as a spreadsheet and Statistix 8 was used for the analysis of all data. Values are presented as the mean $\pm$ the standard deviation. The lidocaine clearance and albumin production trends were calculated as follows: The raw data concentration values were converted to absolute quantities and graphed according to time. Straight lines were fitted to each set of results and the mean and standard deviation of the gradients of these lines were calculated. In the case of the lactate to pyruvate ratios and liver enzyme results, the mean and standard deviations were calculated according to each time interval in each experiment. Flow cytometry results were calculated as above,

and where appropriate, P values ($P<0.05$) were calculated by the 2-tailed Mann-Whitney t-test for possible significant differences.

A.3 Cell culturing, metabolic evaluations and statistics

Daily sampling investigated lactate dehydrogenase (LD), aspartate aminotransferase (AST), glucose, lactate and pyruvate concentrations. These were measured using enzymatic kits. pO_2, pCO_2 and pH were measured on a blood gas machine. The oxygen uptake rate (OUR) was calculated after sampling with the gas supply turned off.

Metabolic clearance/production studies were performed in both dynamic and static configurations as follows: on day 2 D(+)galactose elimination, using gas chromatography mass spectrometry (GC-MS) for detection; on day 3 ammonia detoxification (NH_4Cl) with urea synthesis, using enzymatic methods for detection. On day 4 lidocaine clearance, using LC-MS for detection; and on day 5 albumin production, using a spectrophotometric method. Upon termination on day 7, imaging studies involved either scanning electron microscopy (SEM), to investigate the presence of cells in the foam, or isotopic scanning to examine the seeded-distribution of active hepatocytes in the foam.

For SEM the method was as follows; Circulating medium was replaced with fixative: 2.5% glutaraldehyde in a 0.1 M phosphate buffer (PBS) at pH 7.4. After 30 minutes circulation the foam was removed and sections cut from the inlet, middle and outlet. After washing in PBS buffer these were placed in 1% Osmium tetroxide (OsO_4) for 30 minutes. Following water washes, the samples were dried in ethanol and mounted on aluminium plates. After high pressure CO_2 critical point drying for an hour the samples were gold sputtered and viewed with a JEOL JSM-840 Scanning Electron Microscope.

Radioactive labeling was performed by the active uptake of a 300 μCi dose of ^{99m}Tc-labeled-DISIDA N-(2,6-diisopropylacetanilide)-imino-diacetate which is metabolized only by active hepatocytes. This was injected into the medium and allowed to circulate for 6 minutes. The medium was drained and the circuit washed twice, after which the foam was removed and cut into three radial sections at the inlet, central and outlet portions along the bioreactor axis. These sections were placed on the inverted face of a low energy, high-resolution collimator of an Elscint Apex gamma camera, and scanned for 10 minutes.

Statistics: Microsoft Excel was used for data processing while Statistix 8 was used for analysis. Values are presented as the mean $\pm$ standard deviation. Clearance/production rates were calculated by converting the raw data to absolute quantities and graphing according to time. The gradients of the linear fittings were taken to be the rates. Statistical significance was measured using Student's t test.

Table A.4.1 Modified-HGM cell culture media components

Component	Concentration
Deionized autoclaved H_20	10 L/bottle of DMEM
*Powder DMEM +BSS,glutamine	equivalent for 10 L
$NaHCO_3$	22 g for 10 L equivalent
*Streptomycin-Fungizone	10 ml/L
*Gentamycin sulphate	1 ml/L
*Fetal Calf Serum	100 ml/L
Insulin	mUnits/L
Glucagon	15-20 µg/L
Dexamethazone	67 µg/L
Epidermal Growth factor	20 µg/L
Transferrin (Fe^{2+} saturated)	200 µl/L or 5-6 mg/L
DMSO	1 % v/v
Glucose	2 g/L
Galactose	2 g/L
Nicotinamide	0.610 g/L
Zinc chloride	0.544 mg/L
Zinc sulphate	0.750 mg/L
Cupric sulphate	0.2 mg/L
Manganese sulphate	0.025 mg/L
Sodium selenite	5-6 µg/L

Note: All items except * purchased from Sigma-Aldrich, Johannesburg, South Africa. *'s were purchased from Bio-Whittaker, Adcock Ingram Scientific, Johannesburg, South Africa

Appendix B: *In vivo* study methods

B.1 Anesthesia protocol

Caprofen (Rimadyl ®, 5 mg/kg BW SC) was injected pre-operatively, followed by isoflurane (Safe Line pharmaceuticals) inhalation using a Boyle's isofor inhalation machine with 100 % O_2. Buprenorhine (Temgesic ®, 0.1 ml/100g BW IM) was given at the time of incision. To manage pain post-operatively, carprofen was given once daily with buprenorphine adjusted to 30 % of normal liver weight every 12 hrs. On termination, all animals were euthanased through inhalation of a lethal overdose of isoflurane.

Recovery, pain and toxicity scoring

The National Society for the Protection and Care of Animals (NSPCA) pain and toxicity scoring sheets were completed once daily to assess possible toxicity, pain and humane end-points for the experiments.

Statistics

Microsoft Excel (ver. 2003) was used as a spreadsheet while Statistix (ver. 8, Tallahasee, Fl, USA) was used for data analysis. The mean and standard deviations were calculated for all variables. Non-parametric Wilcoxon rank sum tests, appropriate for small groups, were used to determine the statistical significance of differences between groups.

B.2 Animal preparation

Pathogen-free pigs were purchased from a herd two weeks prior to each experiment to allow for quarantine and acclimatisation. They were housed in environmentally controlled stables (25˚C) with a 12 hr light/dark cycle (University of Pretoria Biomedical Research Centre). Food was composed of a standard pig diet (EPOL) and water until fasting commenced 24 hours prior to each experiment. Energy and electrolytes (Rehidrat, Pfizer) were supplemented during the daytime (08:00-16:00). At 16:00 lorazepam was administered (2 mg IM, Ativan, Aspen) by using a pole-syringe (Dan-Inject) followed by an antibiotic (1g IV, Ceftriaxone, Pharmacare) as intestinal flora prophylaxis. Each pig was hence kept nil per mouth until commencement at 07.00 the following morning.

Anaesthesia protocol

The pigs were immobilized by IM injection of midazolam (0.3 mg/kg, Dormicum, Roche) and ketamine (10 mg/kg, Anaket, Centaur). A 20G IV Teflon catheter (Jelco, Johnson & Johnson) was placed in the ear vein for induction of anesthesia with propofol (3 mg/kg, Diprivan, Astra Zeneca) and intubation with a 7.5 mm endotracheal tube. A nasogastric tube was placed per os to deflate the stomach. After sterile preparation the animal was transferred to the theatre where it was immobilized in the supine position and draped for abdominal surgery. Anaesthesia was maintained with 1.5% isoflurane in an air-oxygen mixture with the aid of a circle rebreathing anaesthetic machine with carbon dioxide absorption (Procare 500, Ohmed, Scientific Group). Fresh gas flow rate was set

at 300 ml/min for oxygen and 600 ml/min for air. Minute volume was maintained with positive pressure ventilation (Ohmeda 7000 Ventilator) to maintain end-tidal carbon dioxide partial pressure in the range of 35-40 mmHg. Intra and postoperative analgesia was supplemented with the lumbar epidural administration of ropivacaine (0.2 ml/kg, Naropin, Astra) and morphine sulphate (0.1 mg/kg, morphine sulphate-Fresenius amps, Fresenius Kabi). Ceftriaxone (1g, Pharmacare) was administered IV as before. Blood volume and blood glucose were maintained with 5% dextrose in a balanced electrolyte solution (Intramed, Ringer Lactate) administered at 10 ml/kg/hr for the duration of anesthesia. All pulse-oximitery (TL-101T, Nihon Kohden, Medical Systems), CO_2 (TG-900P, Nihon Kohden, Medical Systems), electrocardiographic (ECG) (BR-903P, Nihon Kohden, Medical Systems) and electroencephalographic (EEG) electrodes were attached to the animal at this time. Prior to liver devascularization, 500 ml of a gelatin plasma-expander (20 ml/kg IV, gelofusine, B/Braun) was administered IV. This dose was repeated immediately following liver devascularization. Perioperatively, arterial blood pressure was maintained at a mean pressure of between 60-80mmHg (MX 950 Transtar Pressure Transducers, Medex Medical) with the IV infusion of phenylephrine (2-25 µg/kg/min phenylephrine, Covan). Core body temperature was maintained as near as possible to 37.5 degrees C using forced hot air (Bair hugger 505, Augustine Medical).

Catheter placement

Prior to the liver devascularization procedure, an arterial catheter (G16, 115.17 Vigon, Viking Medical) was placed in the common carotid artery for monitoring arterial pressures and blood gases. The external jugular vein was exposed for cannulation with a vascath (CS 15123E, Arrow) and a double lumen venous catheter (CV50688 Fr 7, Arrow) was inserted into the lumen of the internal jugular vein for monitoring central venous pressure (CVP). Positioning was verified after connection of the respective catheters to the monitors. A supra-pubic cystostomy was also performed prior to closing the abdomen using a 10 fg Foleys catheter to monitor urine output

Intensive care

Following surgery the animal was transferred to an intensive care unit (ICU). Continuous ventilation was maintained (40% O_2, tidal volume 10-15 ml/kg) with a post expiratory pressure (PEEP) of 5 mmHg (Ventilator 7200a, Puritan-Bennet). Settings were adjusted hourly according to arterial blood gas (ABG). Sedation was maintained by infusing midazolam (0.3 mg/kg/hr Dormicum, Roche), fentanyl (0.02 mg/kg/hr Fentanyl, Janssen) and pentobarbitone (4 mg/kg/hr Pentobarbitone, 6%, Kyron) with infusion pumps (Modular 3000, Smith's Medical). Boluses of muscular relaxant (0.3 mg/kg/hr Esmeron, Sanofi Synthelabo) were administered IV when necessary. Hemodynamic stability was regulated according to CVP (no less than 14 mmHg) and urine output (2 ml/kg/hr) using fluid boluses including Ringer's lactate (Adcock Ingram) and colloid (Gelofusine, B/Braun). Blood glucose was maintained using 50% glucose (Adcock Ingram) to prevent hypoglycaemia. Dobutamine (2.5-10 µg/kg/min Dobutrex, Eli Lilly) and Phenylephrine (1 µg/kg/min Phenylephrine, Knoll) were titrated to maintain mean arterial blood pressure at a minimum of 60mmHg. Blood potassium (3.4-4.5 mmol/l) and sodium (135-145 mmol/l) concentrations were maintained using Potassium chloride (Adcock Ingram) and Sodium chloride (Adcock Ingram). Heparin was titrated according to activated

clotting time (ACT). Bolus doses (3-5 units/kg) was administered IV until the ACT (as measured by a Hemochron JR, Brittan Health Care) returned to normal (220-250 secs). Body temperature was maintained as above. Intensive care was maintained until the cessation of cardiac function, which was defined as the point of death in this study.

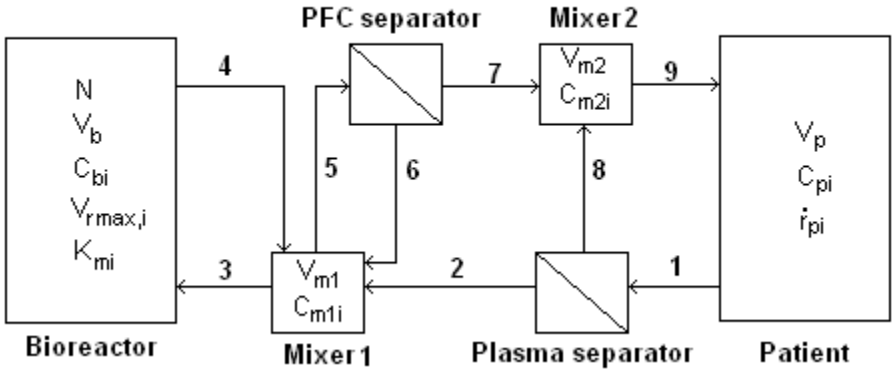
Clinical measurements

Systemic and biochemical indices were measured for the duration of each experiment (table 4.1). Arterial blood pressure and Central venous pressure (CVP) were connected to a calibrated electronic pressure transducer (MX9522, Medex, SSEM). A multiparameter patient monitor (BMM-10-1K, Gambro and BSM 4103K Nihon Kohden) was used to monitor the ECG, pulse rate, CVP, systolic-, diastolic-, mean arterial blood pressure and haemoglobin saturation with the aid of a pulse oximeter probe placed on the tongue. End-tidal CO_2 partial pressure was measured with an in-line sensor placed between the endotracheal tube connection and the breathing circuit. Rectal temperature and ABG measurements were performed hourly while blood biochemical samples were taken four-hourly. Standard [human] laboratory methods were used for these indices. Continuous EEG measurement was performed until termination. A diagrammatic drawing of the brain structure was superimposed on the external bone features and a standardized measuring protocol was adapted from the International 10/20 electrode placement system for humans. Subcutaneous needle electrodes were used for registering activities at the frontal, central, temporal and occipital regions of the brain. A digital recording system was used for EEG monitoring (Medtronic Walter Graphtek PL-EEG, Medtronic) and a software package (Neuro, Galileo NT version 2.31/00, Medtronic) was used for digital spectral Fast Fourier Transform (FFT) analysis. Frequency spectra of 10-second epochs were used in the analysis. In this study we describe only the alpha (8-15 Hz), total delta (0-4 Hz) and relative power values in the frontal and central regions of the brain. All clinical measurements were terminated at death.

Appendix C: The derivation of the compartmental model equations

C.1 System model diagram

The figure below is a simplified representation of the BALSS system connected to a patient, with basic notation and stream (flow-circuit) numbers.



C.2 Model Notation

Symbol	Description	Units
C_{ai}	Concentration of component *i* in stream *a*	mol *i* / m^3
C_{xi}	Concentration of component *i* in compartment *x*	mol *i* / m^3
f_i	Fraction of substrate *i* that is unbound	
g	Ratio of filtrate to feed flow rates for PFC separator	
H_a	Hematocrit in stream *a* = (Volume cellular components)/(Volume plasma + Volume cellular components)	
$K_{m,i}$	Michaelis constant for substrate *i*	mol *i* / m^3
n_{xi}	Number of moles of component *i* in compartment *x*	mol *i*
$\dot{n}_{ai}$	Molar flow rate of component *i* in stream *a*	mol *i* /s
N	Number of hepatocytes in bioreactor	cells
Q_a	Volumetric plasma flow rate of stream *a* (i.e. excluding PFC and cellular blood components)	m^3/s
$Q_{a,pfc}$	Volumetric flow rate of stream *a*, including PFC = $Q_a/(1-\phi_a)$	m^3/s
$Q_{a,ct}$	Volumetric flow rate of stream *a*, including cellular blood components = $Q_a/(1-H_{ct,a})$	m^3/s
r_{xi}	Reaction rate of component *i* in compartment *x*	mol *i* /m³.s
t	Time	s
$V_{max,i}$	Maximal rate of metabolism for substrate *i*	mol *i* /m³.s
V_x	Volume of compartment *x*, excluding PFC and cellular blood component volume	m^3
$V_{x,pfc}$	Volume of compartment *x*, including PFC = $V_x/(1-\phi_x)$	m^3
$V_{x,ct}$	Volume of compartment *x*, including cellular blood components = $V_x/(1-H_x)$	m^3

Greek symbols

ϕ_a	Volume fraction of PFC (perfluorocarbon) in stream *a*

General subscripts

0	Value of variable at time zero
a	Stream *a*
b	Bioreactor
ct	Includes cellular blood components
m1	Mixer 1
m2	Mixer 2
p	Patient
pl	Plasma
ι	Constituent / toxin *i*
x	Compartment *x*

C.3 Input parameters (with typical/indicative values for the UP-CSIR BALSS, where available)

Parameter	**Description**	**Typical value**	**Units**
$C_{bi,0}$	Starting concentration of component *i* in bioreactor	0	mol/m^3
$C_{m1i,0}$	Starting concentration of component *i* in mixer *m1*	0	mol/m^3
$C_{m2i,0}$	Starting concentration of component *i* in mixer *m2*	0	mol/m^3
$C_{pi,0}$	Starting concentration of component *i* in patient	0.07	mol/m^3
f_i	Fraction of total substrate *i* that is unbound	1	
g	Filtrate to feed flow rate for PFC separator	0.025	
H_8	Hematocrit of plasma separator concentrate	0.8	
H_p	Hematocrit in patient	0.5	
K_{mi}	Michaelis constant for substrate *i*	20.86	mol *i* / m^3
N	Number of hepatocytes in bioreactor	$1x10^{10}$	cells
$Q_{1,ct}$	Rate of withdrawal of blood from patient	$2x10^{-6}$	m^3/s
$Q_{3,pfc}$	Flow rate of plasma-PFC blend into bioreactor	$8.33x10^{-6}$	m^3/s
r_{pi}	Rate of reaction of component *i* in patient	$3.56x10^{-8}$	mol/m^3.s
$V_{b,pfc}$	Volume of bioreactor	0.0003	m^3
$V_{r\max}$	Maximum metabolic rate of hepatocytes	$3.1x10^{-11}$	mol/s.cell
$V_{m1,pfc}$	Volume of mixer *m1*	0.00065	m^3
$V_{m2,ct}$	Volume of mixer *m2*	0.0001	m^3
$V_{p,ct}$	Volume of blood in patient	0.004	m^3
ϕ_3	Volume fraction PFC in stream 3	0.1	

C.4 Output parameters

$C_{pi}, C_{bi}, C_{m1i}, C_{m2i}$

C.5 Variables that influence the outputs,

g
f_i
N
V_p, V_b, V_{m1}, V_{m2}
$C_{pi,0}$
$V_{\max,i}, K_{mi}, r_{pi}$
$Q_{1,ct}, Q_3$

C.6 Basic assumptions

1. Constituent *i* is well mixed in the bioreactor (*b*), patient (*p*), mixer 1 (*m1*) and mixer 2 (*m2*) (i.e. these vessels are modeled as continuously stirred tank reactors).
2. Both plasma and PFC separators have 100% separation efficiency (i.e. no cellular blood components pass into stream 2, and no PFC (perfluorocarbon) emulsion droplets pass into stream 7).
3. Volumes of lines and separators are negligible.
4. Separators do not differentially separate plasma constituents (i.e. filtrate and concentrate have the same plasma constituent concentrations).
5. Changes in stream volumetric flow rates due to reactions are negligible.
6. PFC does not absorb any plasma components/constituents in significant quantities.
7. Rate of production in patient ($r_{pi}V_p$) is the net rate (i.e. production by body – clearance by body – excretion by body).
8. Bioreactor clearance rates are determined by cell number and by toxin concentration.
9. Mixer 2 is a combination of the two physical reservoirs (the plasma and blood reservoirs).

C.7 Flow rates

The total blood flow rate of stream 1, $Q_{1,ct}$, is an input parameter. The flow rate of stream 8, $Q_{8,ct}$, can be calculated from the known hematocrit (H) levels in streams 1 and 8, where the definition of hematocrit in this document is defined as the volume fraction of cellular components in the total blood stream:

$$Q_{8,ct} = \frac{H_1}{H_8} Q_{1,ct}$$
$$Q_8 = \frac{(1-H_8)}{(1-H_1)} \frac{H_1}{H_8} Q_1 \qquad \text{(C.1)}$$

where $H_1=H_p$

The flow rate of stream 2:

$$Q_2 = Q_1 - Q_8 \tag{C.2}$$

The return flow rate of blood to the patient must be equal to the flow rate of blood extracted from the patient:

$$Q_9 = Q_1 \tag{C.3}$$

The flow rate of stream 7 can now be calculated:

$$Q_7 = Q_9 - Q_8 \tag{C.4}$$

The exit flow rate from the bioreactor must be equal to the inlet flow rate:

$$Q_4 = Q_3 \tag{C.5}$$

The ratio of filtrate flow rate to inlet flow rate for the PFC separator, g, is given by:

$$\begin{aligned} g &= Q_7/Q_5 \\ \therefore Q_5 &= Q_7/g \end{aligned} \tag{C.6}$$

The concentrate flow rate from the PFC separator is now given by:

$$\begin{aligned} Q_6 &= Q_5 - Q_7 \\ Q_6 &= Q_7(1/g - 1) \end{aligned} \tag{C.7}$$

C.8 Concentrations

The number of moles of component i in compartment x is given by:

$$n_{xi} = C_{xi}V_x \tag{C.8}$$

The molar flow rate of component i in stream a is given by:

$$\dot{n}_{ai} = C_{ai}Q_a \tag{C.9}$$

Because of the assumption of good mixing, the concentration of a constituent in the exit streams from any vessel is equal to the concentration of the same constituent in the vessel at any given time:

$$\begin{aligned} C_{1i} &= C_{pi} \\ C_{9i} &= C_{m2i} \\ C_{3i} &= C_{5i} = C_{m1i} \\ C_{4i} &= C_{bi} \end{aligned} \tag{C.10}$$

Because no concentration changes occur in the separators, separator exit concentrations are equal to separator inlet concentrations:

$$\begin{aligned} C_{2i} &= C_{8i} = C_{1i} \\ C_{6i} &= C_{7i} = C_{5i} \end{aligned} \tag{C.11}$$

C.9 Molar balances

Taking a mole balance for component *i* over the patient:

$$\frac{dn_{pi}}{dt} = \text{production - consumption + in - out}$$
$$\frac{d\left(V_p C_{pi}\right)}{dt} = r_{pi}V_p - 0 + C_{9i}Q_9 - C_{1i}Q_1 \tag{C.12}$$

Substituting from equation (C.10), and rearranging:

$$V_p \frac{dC_{pi}}{dt} = r_{pi}V_p + C_{m2i}Q_9 - C_{pi}Q_1$$
$$\frac{dC_{pi}}{dt} = r_{pi} + \frac{Q_9}{V_p}C_{m2i} - \frac{Q_1}{V_p}C_{pi} \tag{C.13}$$

Similarly, taking a mole balance over the bioreactor:

$$\frac{dn_{bi}}{dt} = \text{production - consumption + in - out}$$
$$\frac{d\left(V_b C_{bi}\right)}{dt} = 0 + r_{bi}V_b + C_{3i}Q_3 - C_{4i}Q_4 \tag{C.14}$$

Substituting from equation (C.10) and rearranging:

$$V_b \frac{dC_{bi}}{dt} = r_{bi}V_b + C_{m1i}Q_3 - C_{bi}Q_4$$
$$\frac{dC_{bi}}{dt} = \frac{Q_3}{V_b}C_{m1i} - \frac{Q_4}{V_b}C_{bi} + r_{bi} \tag{C.15}$$

Taking a mole balance over mixer *m1*:

$$\frac{dn_{m1i}}{dt} = \text{production - consumption + in - out}$$
$$\frac{d\left(V_{m1}C_{m1i}\right)}{dt} = 0 - 0 + C_{2i}Q_2 + C_{6i}Q_6 + C_{4i}Q_4 - C_{3i}Q_3 - C_{5i}Q_5 \tag{C.16}$$

Substituting from equations (C.10) and (C.11), and rearranging:

$$V_{m1}\frac{dC_{m1i}}{dt} = C_{pi}Q_2 + C_{m1i}Q_6 + C_{bi}Q_4 - C_{m1i}Q_3 - C_{m1i}Q_5$$
$$\frac{dC_{m1i}}{dt} = \frac{Q_2}{V_{m1}}C_{pi} + \frac{Q_4}{V_{m1}}C_{bi} + \frac{(Q_6 - Q_3 - Q_5)}{V_{m1}}C_{m1i} \tag{C.17}$$

Taking a mole balance over mixer *m2*:

$$\frac{dn_{m2i}}{dt} = \text{production - consumption + in - out}$$
$$\frac{d\left(V_{m2}C_{m2i}\right)}{dt} = 0 - 0 + C_{8i}Q_8 + C_{7i}Q_7 - C_{9i}Q_9 \qquad \text{(C.18)}$$

Substituting from Equations (C10) and (C11), and rearranging:

$$V_{m2}\frac{dC_{m2i}}{dt} = C_{pi}Q_8 + C_{m1i}Q_7 - C_{m2i}Q_9$$
$$\frac{dC_{m2i}}{dt} = \frac{Q_8}{V_{m2}}C_{pi} + \frac{Q_7}{V_{m2}}C_{m1i} - \frac{Q_9}{V_{m2}}C_{m2i} \qquad \text{(C.19)}$$

C.10 Rates of production/clearance

The production rate (r_{pi}) of component i in the patient is assumed to be constant. The rate of clearance/metabolism of component *i* in the bioreactor can be approximated using the Michaelis-Menten relationship [91]:

$$\text{Rate of metabolism} = r_{bi} = \frac{V_{\max,i}C_{bi}f_i}{K_{mi} + C_{bi}f_i} \qquad \text{(C.20)}$$

When $C_{m1i}.fi >> K_{mi}$, then the rate of metabolism = $V_{\max,i}$. When $C_{m1i}.f_i << K_{mi}$, then the rate of metabolism = $V_{\max,i}.f_i.C_{m1i}/K_{mi}$. The Michaelis-Menten parameters ($V_{\max,i}$ and K_{mi}) can be determined from a single compartment bolus experiment by rewriting Equation (A.20), as follows:

$$\frac{dC_i}{dt} = r_i = -\frac{V_{\max,i}C_i f_i}{K_{mi} + C_i f_i}$$
$$-\frac{dt}{dC_i} = -\frac{1}{r_i} = \frac{K_{mi}}{V_{\max,i}C_i f_i} + \frac{1}{V_{\max,i}} \qquad \text{(C.21)}$$

Thus a plot (called a Lineweaver-Burk plot) of $1/r_i$ vs. $1/C_i$ should yield a straight line with y-intercept $1/V_{\max,i}$ and gradient $K_{mi}/V_{\max,i}f_i$.

The $V_{\max}$ value determined from the plot is dependent on enzyme concentration (e.g. if enzyme concentration doubles, $V_{\max}$ doubles), while K_m is not [91]. If one assumes that enzyme concentration is directly proportional to hepatocyte 'concentration' (i.e. hepatocytes per reactor volume), then one can calculate a reduced maximum reaction rate ($V_{r\max}$, with units mol/s.cell) from the experimentally determined $V_{\max}$ value as follows:

$$V_{r\max} = \frac{V_{\max}V_b}{N}$$
$$\text{and } V_{\max} = \frac{V_{r\max}N}{V_b} \qquad \text{(C.22)}$$

This reduced maximum reaction rate can now be used to estimate V_{max} for different reactor sizes and hepatocyte loadings.

Using equation (C.20), equation (C.15), can now be rewritten as:

$$\begin{aligned} V_b \frac{dC_{bi}}{dt} &= r_{bi} V_b + C_{m1i} Q_3 - C_{bi} Q_4 \\ V_b \frac{dC_{bi}}{dt} &= -\frac{V_{\max,i} C_{bi} f_i V_b}{K_{mi} + C_{bi} f_i} + C_{m1i} Q_3 - C_{bi} Q_4 \\ \frac{dC_{bi}}{dt} &= -\frac{V_{\max,i} C_{bi} f_i}{K_{mi} + C_{bi} f_i} + \frac{Q_3}{V_b} C_{m1i} - \frac{Q_4}{V_b} C_{bi} \end{aligned} \tag{C.23}$$

C.11 Summary of equations and parameters

$$\begin{aligned} \frac{dC_{pi}}{dt} &= r_{pi} + \frac{Q_9}{V_p} C_{m2i} - \frac{Q_1}{V_p} C_{pi} \\ \frac{dC_{bi}}{dt} &= -\frac{V_{\max,i} C_{bi} f_i}{K_{mi} + C_{bi} f_i} + \frac{Q_3}{V_b} C_{m1i} - \frac{Q_4}{V_b} C_{bi} \\ \frac{dC_{m1i}}{dt} &= \frac{Q_2}{V_{m1}} C_{pi} + \frac{Q_4}{V_{m1}} C_{bi} + \frac{(Q_6 - Q_3 - Q_5)}{V_{m1}} C_{m1i} \\ \frac{dC_{m2i}}{dt} &= \frac{Q_8}{V_{m2}} C_{pi} + \frac{Q_7}{V_{m2}} C_{m1i} - \frac{Q_9}{V_{m2}} C_{m2i} \end{aligned} \tag{C.24}$$

These equations are supported by the following set of algebraic equations:

$$\begin{aligned} Q_8 &= \frac{(1-H_8)}{(1-H_1)} \frac{H_1}{H_8} Q_1 \\ Q_2 &= Q_1 - Q_8 \\ Q_9 &= Q_1 \\ Q_7 &= Q_9 - Q_8 \\ Q_4 &= Q_3 \\ Q_5 &= Q_7 / g \\ Q_6 &= Q_7 (1/g - 1) \\ C_{1i} &= C_{pi} \\ C_{9i} &= C_{m2i} \\ C_{3i} &= C_{5i} = C_{m1i} \\ C_{4i} &= C_{bi} \\ C_{2i} &= C_{8i} = C_{1i} = C_{pi} \\ C_{6i} &= C_{7i} = C_{5i} = C_{m1i} \end{aligned} \tag{C.25}$$

Calculation of flow rates and volumes from input parameters:

$$\begin{aligned} Q_1 &= Q_{1,ct}\left(1-H_p\right) \\ Q_3 &= Q_{3,pfc}\left(1-\phi_3\right) \\ V_p &= V_{p,ct}\left(1-H_p\right) \\ V_b &= V_{b,pfc}\left(1-\phi_3\right) \\ V_{m1} &= V_{m1,pfc}\left(1-\phi_3\right) \\ V_{m2} &= V_{m2,ct}\left(1-H_{ct,p}\right) \end{aligned} \tag{C.26}$$

Calculation of PFC volume fraction in stream 6:

$$\begin{aligned} &\phi_5 Q_5 = \phi_6 Q_6 + \phi_7 Q_7 \\ &\text{But } \phi_7 = 0 \\ &\therefore \phi_5 Q_5 = \phi_6 Q_6 \\ &\phi_6 = \frac{Q_5}{Q_6}\phi_5 \end{aligned} \tag{C.27}$$

Appendix D: *On line* model sensitivity and verification

Table D.1 Numerical associations in and between classes

Association	Variable 1	Variable 2	Pearson correlation > 0.4	Spearman correlation > 0.3	Comments
	ave_pH	rLactate	-0.71	-0.47	
	ave_pH	ave_pCO_2	-0.67	-0.47	sources of change in pH
	ave_pH	ave_HCO_3	+0.46	+0.58	
	ave_Pulse	rNa+	-0.61	-0.33	electrolyte effect
	rK+	rCreatinine	+0.87	+0.64	kidney indices related
	rCreatinine	Urine_Tot	-0.72	-0.70	
	rBilirubin	rALP	+0.46	+0.59	
	rBilirubin	rALT	+0.73	+0.78	
	rBilirubin	rAST	+0.66	+0.74	
	rBilirubin	rLD	+0.48	+0.73	Liver indices related
Internal	rAmmonia	rBcAA/AroAA	+0.79	-0.39	
	rAmmonia	rAST	+0.53	+0.56	
	rAmmonia	rLD	+0.74	+0.37	
	Fr_a/d_fr	Pw_a/d_fr	-0.47	-0.57	EEG frequency and power
	rPT	rAPTT	-0.49	-0.30	
	rPT	rAntiThrombin	+0.79	+0.68	
	rPT	rFibrinogen	+0.46	+0.31	
	rFibrinogen	rAntiThrombin	+0.81	+0.61	Coagulation indices
	rFactor II	rFactor VII	+0.71	+0.66	
	rFactor VII	rFactor X	+0.69	+0.52	
	rFactor II	rFactor X	+0.44	+0.40	
	rAmmonia	Fr_a/d_fr	-0.79	-0.54	Encephalopathy
	rAmmonia	Fr_a/d_ct	-0.42	-0.36	and the liver
	rAmmonia	Pw_a/d_fr	+0.38	+0.43	
	rBcAA/AroAA	Fr_a/d_fr	+0.90	+0.75	Encephalopathy
	rBcAA/AroAA	Fr_a/d_ct	+0.61	+0.43	and *Fischer's ratio
	rBcAA/AroAA	Pw_a/d_fr	-0.47	-0.43	
	rGlutamine	Fr_a/d_fr	-0.61	-0.46	Encephalopathy
	rGlutamine	Fr_a/d_ct	-0.40	-0.57	and glutamine
	rGlutamine	Pw_a/d_fr	0.92	+0.93	
External	rLactate	Fr_a/d_fr	-0.96	-0.64	Encephalopathy
	rLactate	Fr_a/d_ct	-0.86	-0.89	and lactate
	rLactate	Pw_a/d_fr	+0.47	+0.64	
	rPyr	Fr_a/d_ct	-0.57	-0.39	
	rPyr	Pw_a/d_fr	+0.48	+0.64	
	rCreatinine	rGlutamine	+0.61	+0.43	Kidney function and *AAs
	rCreatinine	rPT	+0.43	+0.72	Coagulopathy
	rCreatinine	rFibrinogen	-0.44	-0.39	and the kidneys
	rCreatinine	rAmmonia	+0.78	+0.35	Liver and kidneys
	Urine_Tot	rHkt	+0.59	+0.53	The effect of fluids
	Fluids_Tot	rHkt	+0.52	+0.46	on hematocrit
	rHb	rHkt	+0.81	+0.68	

Notes: 1. Inclusion in the table required a Pearson coefficient > 0.4 and a Spearman coefficient> 0.3.

2. prefixes: r = rate of change over time, and ave_ = average over time. 3. All variables with Fr_a/d were for indices of electro-encephalograms (EEG), which were measured in the animal experiments, but have shown no prognostic value. BcAA/AroAA = Fischer's ratio, where AAs = amino acids (see section 4.2).

D.2 First-order assumptions

The assumption of the linearity over time of the composing first order equations was investigated by determining the best-fit linear equation for each data for all variables. A mean R^2 value, the numerical square of the Pearson coefficient (and the proportion of the variance in the dependent variable attributable to the variance in each independent variable) was calculated for all variables (figure D.2.1). The majority of the variables had R^2 values above 0.5 (Pearson coefficients > 0.7) which indicated that they mostly linear and justified the numerical design of the model. Of interest was a strong correlation between the variables that exhibited the greatest change, that is, the highest P values (table 4.2.2) and those that were most linear over time. Variables that had R^2 values < 0.5, but which were still felt to possess some prognostic value were weighted to relatively decrease their contribution to the eventual prediction. Specifically, a percentile weight was used related to their linearity. In general, the variables used for prediction during the surgical interval (T<0) had lower R^2 values (were less linear) than those used during the ICU period (T>0). This validated the use of a larger number of variables in the T<0 period, each with relatively less weight than in the T>0 period. It was also expected that the T<0 part would be less accurate than the T>0 part.

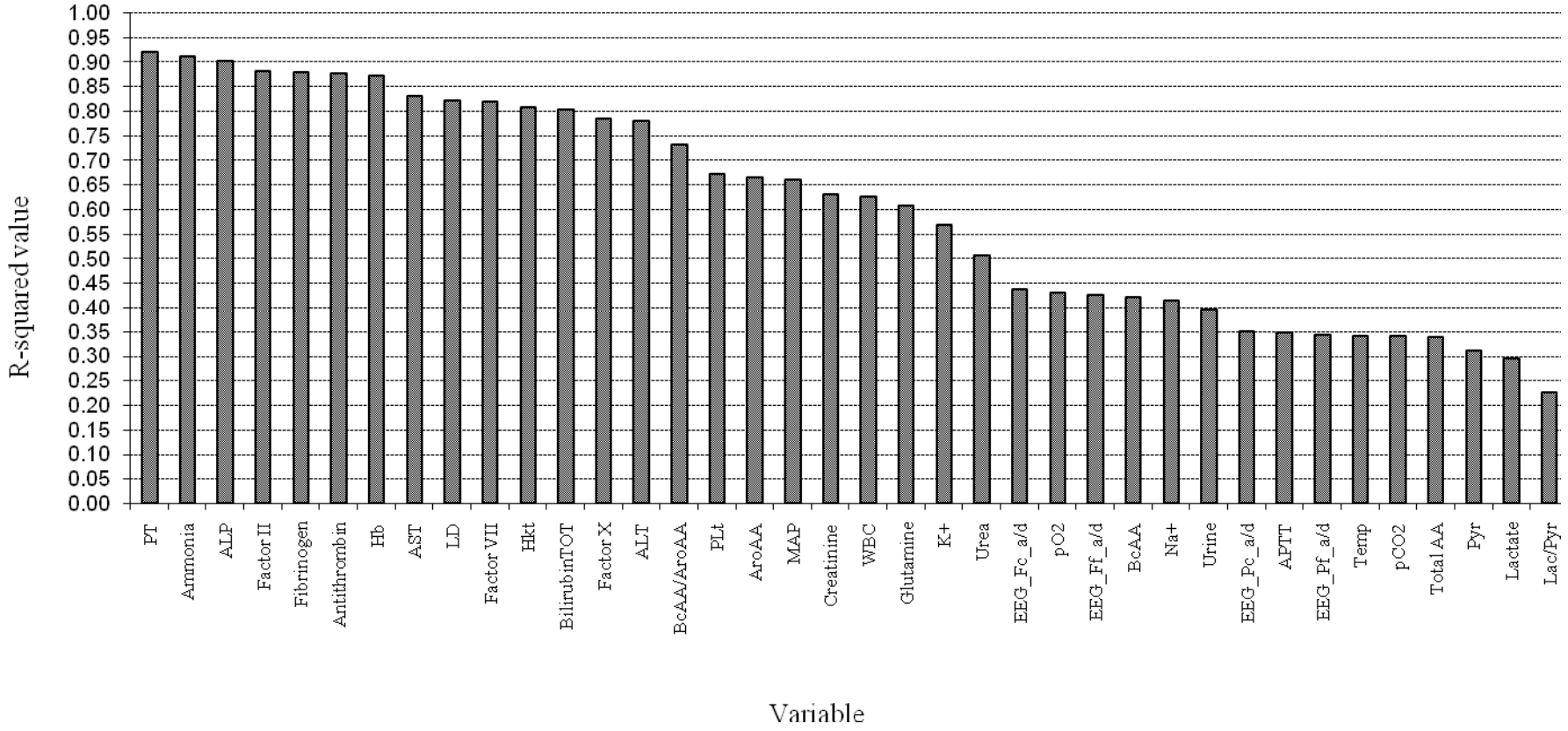


Figure D.2.1 Linearity of variable trends. The majority of measured variables demonstrated an R^2 value above magnitude 0.5.

D.3 Model sensitivity

1. Tornado diagrams [285] function as a macro in Excel and require the specification of a high and low value for each of the input variables of the model. The output is then displayed in terms of each of the composing variables' ranges about the mean predicted value. Thus, the dependence of the model's output on each of the inputs is visible. i.e. the larger the particular input variable's range about the summated mean predicted value, as determined by the weight appropriated to that variable, the greater the influence that variable has on the model's output. The data range used for the diagrams below was that measured in practice (section 5.2). The method assumes the Gaussian normality of the input populations. Only a selection of the BI Tornado diagrams are presented below:

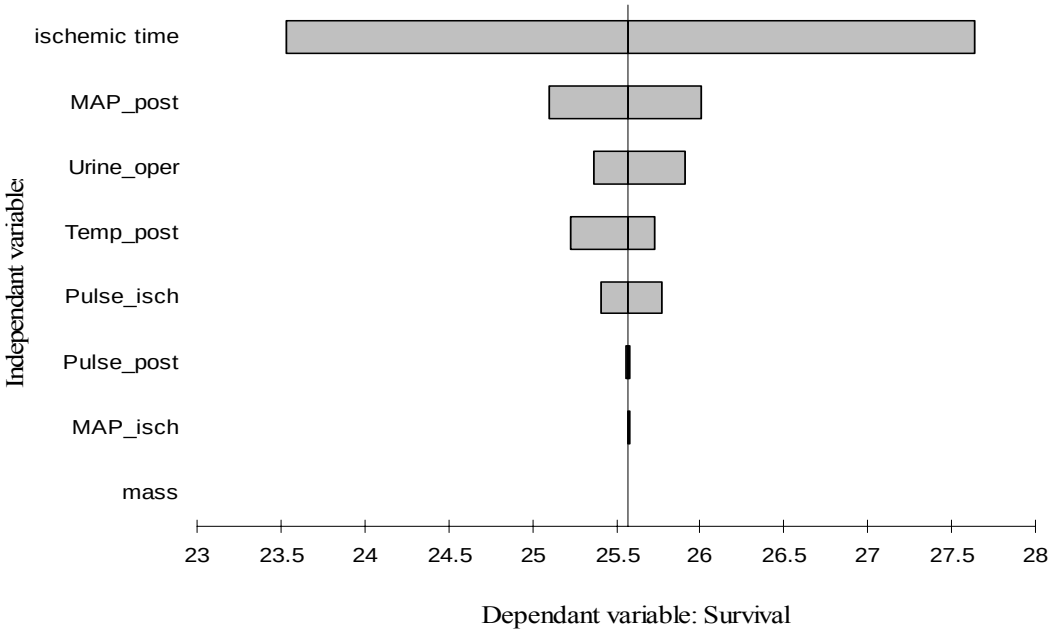


Figure D.3.1 Tornado diagram for PI model (T<0)
Note: The Ischemic time clearly has the greatest impact on Survival during the surgical interval. Output sensitivity is determined by the weight appropriated to each variable.

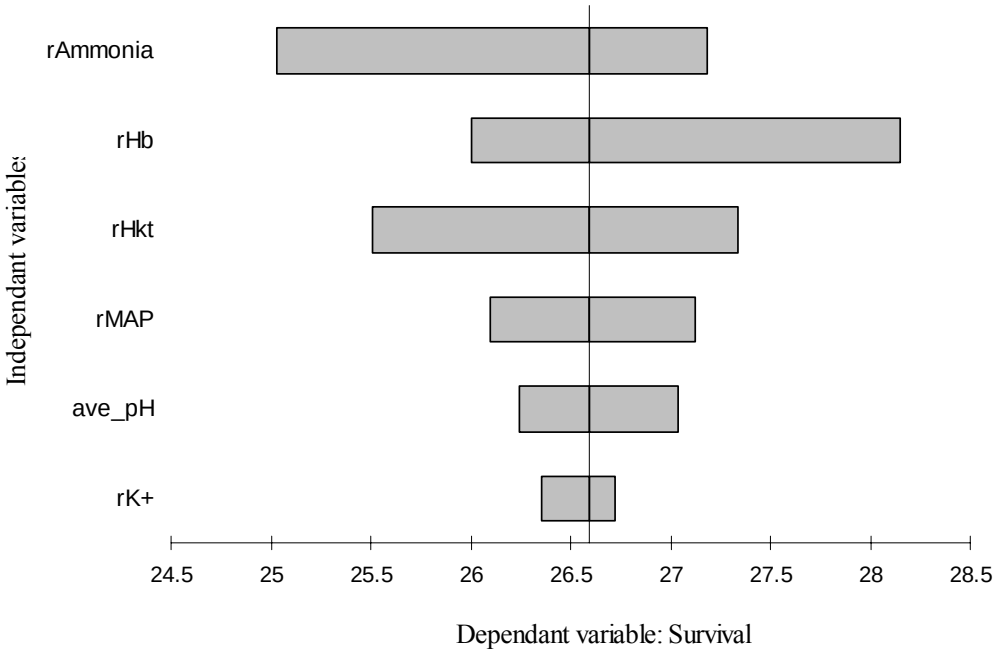


Figure D.3.2 Tornado diagram for PI model (T>0)
Note: The rate of increase of blood Ammonia most strongly impacted Survival after surgery. Output sensitivity is determined by the weight appropriated to each variable.

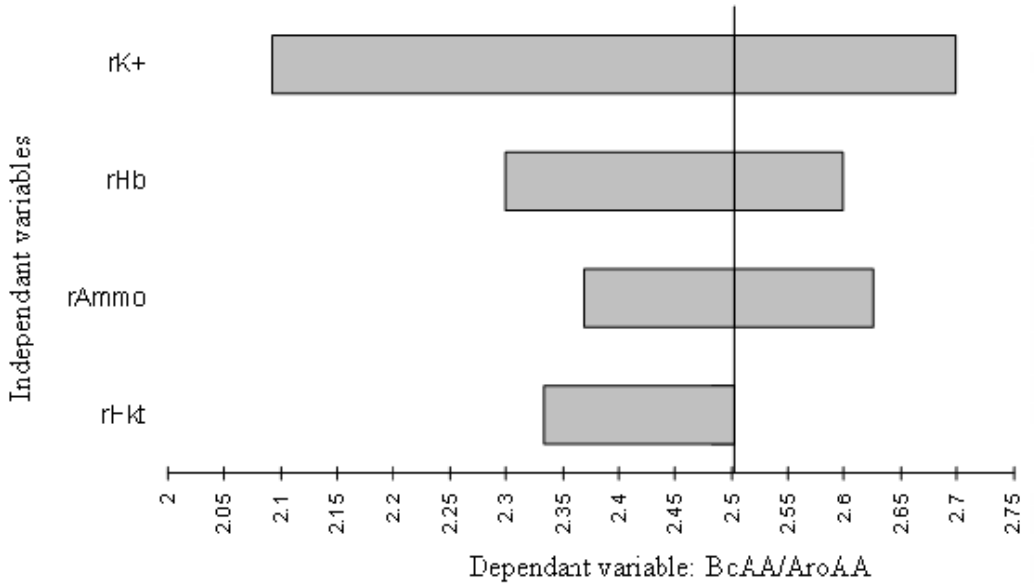


Figure D.3.3 BI Model Sensitivity for BcAA/AroAA (BI) (at 12 hrs)

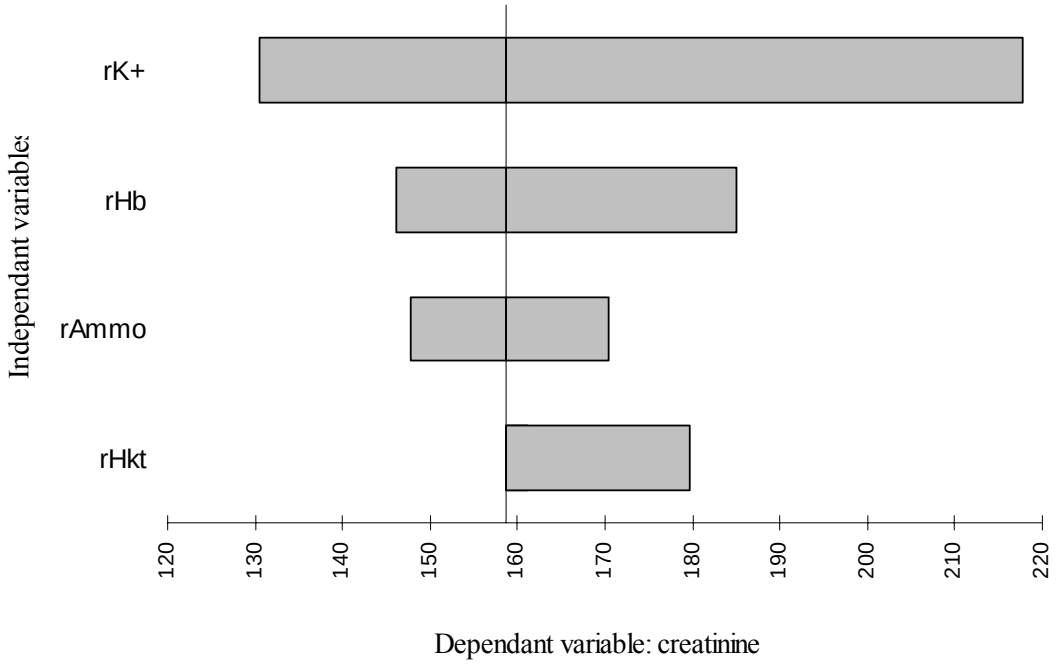


Figure D.3.4 BI Model sensitivity for creatinine (at 12 hrs)

2. Monte Carlo simulation

This procedure [285-287] was used to determine the sensitivity of the model's outputs to generated random numbers as inputs. The model was programmed into an Excel spreadsheet then 1000 random numbers, parameterized about the measured mean and standard deviation for each variable, were generated. This data was used as input to the model. Output sensitivity was determined individually and in combination. i.e. either one variable was randomized independently while retaining all other variables on their mean values, or all variables were randomized simultaneously, followed by the summation of the results. This method of analysis assumes normality in the input data. The latter assumption was also tested by using the same procedure but with uniformly distributed populations of generated numbers. The results were then graphically projected:

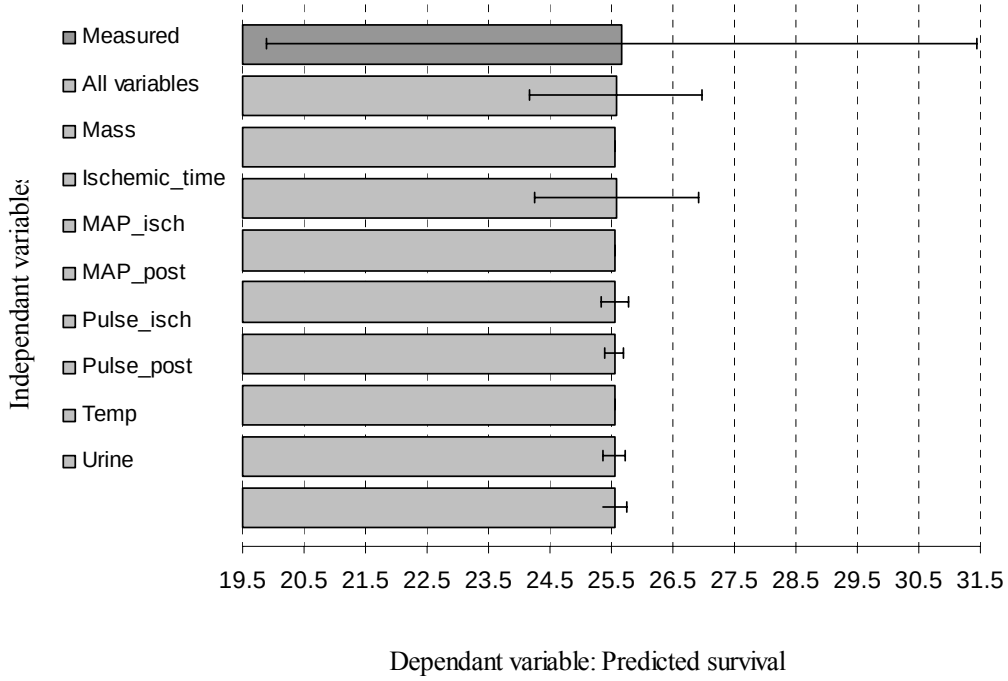


Figure D.3.5 PI Model (T<0) prediction variation using normal distributions (N=1000) of independent variables. The majority of variation in model output originated with the Ischemic_time. All input variables individually very closely approximated the measured mean.

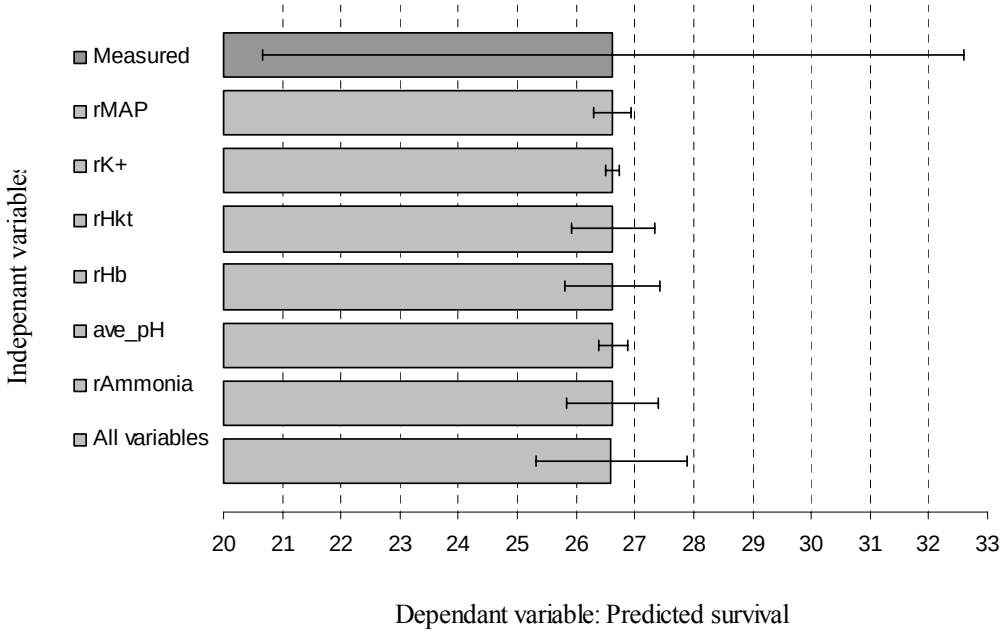


Figure D.3.6 PI Model (T>0) prediction variation using normal distributions [N=1000] of independent variables (T>0). All inputs very closely estimated the measured mean. There was a similar amount of variation in prediction from rAmmonia, rHkt and rHb.

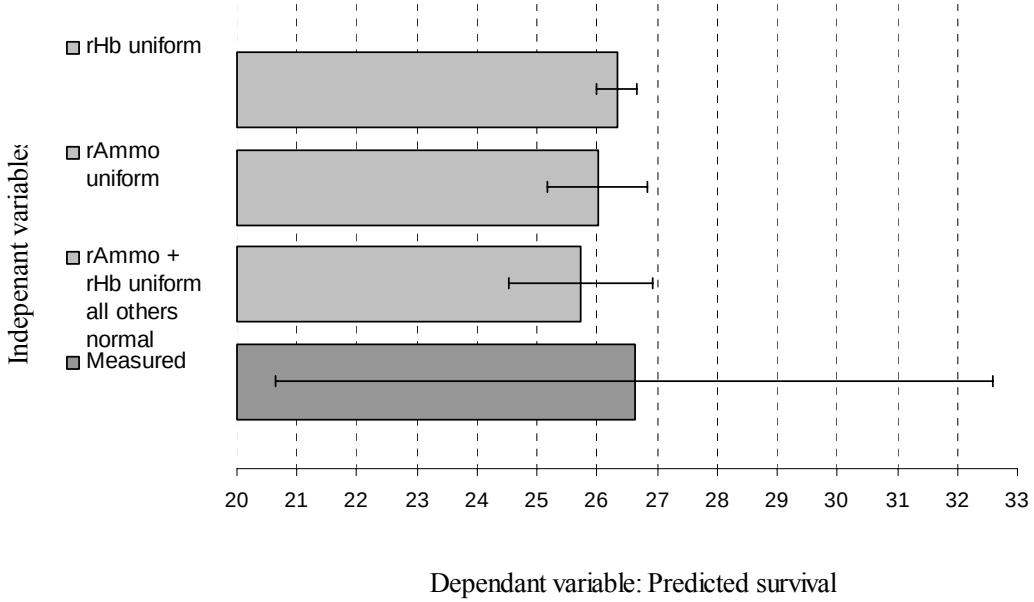


Figure D.3.7 PI Model (T>0) prediction variation using uniform distributions (N=1000) of non-Gaussian variables. Survival was slightly underestimated when the distributions were uniform as opposed to normal.

D.4 Assumptions of normality

The measured raw data for the input variables was tested for normality using Shapiro-Wilk tests with P(W) values as are available in Statistix 8 (table 5.2.7). Despite the small population sizes, it was only in the derived variables rHb and rAmmo (PI model) that normality was excluded in the P(W) values, using the population from which the model's equations had been derived. To examine the effect that non-normal distributions would have on model predictions, the inputs in question were also randomized using uniform distributions. The duration of survival was marginally underestimated in the PI.

Table D.4.1 Normality of independent variables in the PI

Variable	Time period	Shapiro Wilk W-value	P(W) value	Number of cases
Survival		0.8834	0.1705	9
Body weight		0.9485	0.6738	9
Ischemic		0.8502	0.0749	9
time		0.9082	0.3414	8
MAP_isch	**T<0**	0.9767	0.9454	9
MAP_post		0.9191	0.4224	8
Pulse_isch		0.9737	0.9257	8
Pulse_post		0.8442	0.0644	9
Temp_post		0.9063	0.2906	9
Urine_oper				
Survival		0.8925	0.2471	8
rMAP		0.9572	0.9353	8
ave_pH		0.9816	0.9703	8
rK+		0.8727	0.1602	8
rHkt	**T>0**	0.8769	0.1758	8
rHb		0.6765	0.0012	8*
rHb		0.6204	0.0001	12†
rAmmonia		0.7065	0.0027	8*
rAmmonia		0.8200	0.0160	12†

Notes:
1. As the W-value approaches 1, the distribution approaches normality.
If the P(W) value is < 0.05, normality may not be assumed.
2. In the T<0 part of the model all distributions indicated normality.
3. In the T>0 part of the model, only in rHb and rAmmo could normality not be assumed based on the P(W) values in both sets of data.
* Indicates the data sets (N=8) from which the model was initially defined.
† Indicates all the data sets measured (N=12).
4. The effect of non-normal distributions on model prediction variation was tested by means of employing uniform distributions for the above variables (figure 5.2.10).
5. The BI is derived from independent variables present in the T>0 part of the PI. The nature of the distributions of those variables will thus also determine prediction variation in the BI.

Table D.5.1 The number of independent variables used to calculate each biochemical in the BI

	Number of independent variables			
	4	**3**	**2**	**1**
Variable	BcAA/AroAA Fibrinogen Creatinine	ALP	Glutamine Antithrombin LD	Bilirubin PT Factor II, VII, X Factor AST ALT Urea

Notes:
1. The greater the number of weighted input variables the greater the likelihood of prediction accuracy.
2. Due to being determined by only one independent variable, the clotting factors and two of the liver enzymes, are unlikely to be predicted with great accuracy. Unfortunately, at the time of writing, there were no *on-line* sensors for these variables.

D.6 Model Verification methods and results

D.6.1 ANOVA

a. A statistical mean and standard deviation for all measured and predicted values was calculated (columns 4 and 5, table D.6.1). From this a percentage deviation of each predicted mean and standard deviation from each measured mean and standard deviation was calculated (column 6). Bearing in mind the large numerical range of measurement in the biochemical variables it was found that the predicted standard deviations about each mean differed to a greater extent than that in the measured. The greatest error was found in Factor X with a – 4.291 % difference. The percentage error for urea was 280.936%, and those for creatinine and glutamine were also unacceptably large.

b. An ANOVA comparison, using a 'single factor without replication' method on a 0.05 confidence level, was drawn between all predicted and measured populations. The variance in the predicted (above) and measured (below) populations was similar (column 7). In general, the variance in the predicted population was greater than in the measured, with the largest differences in urea, glutamine and creatinine once again. The mean square values (column 8) indicated that sources of variation were less between populations than within them. F-ratios were uniformly smaller than F-crit values, indicating that the differences were best explained by chance. The confidence (P) values (column 10) were mostly above 0.8, with the exception of fibrinogen, AST and LD, whose P values were below 0.55. However, all were far above 0.05, thus, the null hypothesis was rejected. Thus, it was not possible to detect a significant difference between the two populations in any of the parts of the model.

Table D.6.1 ANOVA results for the PI and BI model/s (highlighted variables were discarded)

1. Model Output	2. Pearson Correlation coefficient: predicted to measured	3. Output correction formula	4. Measured: Mean Std dev	5. Predicted: Mean Std dev	6. Percentage deviation of predicted (mean & std dev) from measured	7. Variance: Predicted over Measured.	8. Mean Square (MS): between & within grps.	9. F value & F-crit value.	10. P value (α = 0.05)
Prognosis T<0	0.733	y=0.1913x+20.636	24.88 5.64	25.07 7.69	0.14 4.24	59.08 31.84	0.15 45.46	0.003 4.60	0.956
Prognosis T>0	0.864	y=0.2823x+19.109	26.63 5.98	26.63 6.92	0.001 2.39	47.82 35.70	$5.33x10^{-6}$ 41.76	$1.28x10^{-7}$ 4.60	0.999
BcAA/AroAA	0.811	y=0.6624x+1.3662	1.98 0.75	2.03 0.90	1.27 45.88	0.86 0.57	$1.94x10^{-8}$ 0.72	$2.71x10^{-8}$ 4.03	0.999
BilirubinTOT	0.864	y=1.27x +3.9589	12.41 7.49	13.30 9.24	-0.91 7.28	8.34 56.07	13.74 71.13	0.19 3.98	0.662
Fibrinogen	0.962	y=1.1821x-0.8357	1.96 0.71	1.72 0.74	2.12 27.84	0.55 0.51	0.59 0.53	1.11 4.07	0.297
PT	0.945	y=0.8624x+4.4398	15.39 5.24	16.12 6.57	-0.41 4.31	43.14 27.44	8.82 35.76	0.25 3.99	0.621
AntiThrombin	0.841	y= 0.6329x +28.5	65.98 20.18	65.98 23.99	0.04 1.21	575.56 407.13	$1.11x10^{-4}$ 491.35	$2.26x10^{-7}$ 4.01	0.999
Factor II	0.847	y=0.7741x+8.3169	32.60 13.86	32.60 16.36	-0.03 2.46	267.50 192.09	$1.75x10^{-6}$ 229.8	$7.62x10^{-9}$ 3.99	0.999
Factor VII	0.837	y=0.6739x+14.753	30.35 19.40	30.35 23.18	-0.38 3.83	537.21 376.47	$6.82x10^{-5}$ 456.84	$1.49x10^{-7}$ 3.99	0.999
Factor X	0.786	y=0.6641x+24.718	28.96 27.11	28.96 34.51	-4.29 22.40	$1.19x10^{3}$ 735.03	$3.56x10^{-5}$ 962.96	$3.69x10^{-8}$ 3.99	0.999
ALP	0.747	y=0.4547x+170.63	330.65 260.53	344.56 348.78	-0.10 0.47	$1.22x10^{5}$ $6.79x10^{4}$	$3.39x10^{3}$ $9.56x10^{4}$	0.04 3.98	0.851
AST	0.810	y= 0.763x +1306	1521.16 2025.67	1871.04 2627.43	-0.51 1.13	$6.90x10^{6}$ $4.10x10^{6}$	$2.04x10^{6}$ $5.61x10^{6}$	0.36 3.99	0.549
LD	0.800	y=0.7057x+949.87	1673.18 1545.16	2020.51 2230.23	-0.03 0.11	$4.97x10^{6}$ $2.39x10^{6}$	$2.08x10^{6}$ $3.74x10^{6}$	0.56 3.98	0.459
ALT	0.729	y=0.5878x+88.486	120.0 113.81	126.88 151.95	-0.25 1.69	$2.31x10^{6}$ $1.30x10^{4}$	814.64 $1.83x10^{4}$	0.05 3.98	0.833
Creatinine	0.522	y=0.4318x+82.663	129.55 53.27	129.55 101.95	0.15 1.72	$1.04x10^{4}$ $2.84x10^{3}$	$3.74x10^{-4}$ 6615.82	$5.65x10^{-8}$ 3.99	0.999
Urea	0.164	y= 0.074x +2.391	2.24 0.87	2.31 5.17	-3.66 280.94	26.70 0.75	0.09 13.91	$6.46x10^{-3}$ 13.91	0.936
Glutamine	0.463	y= 0.267x +178.8	207.68 96.39	207.71 207.95	-0.05 1.10	$4.32x10^{4}$ $9.28x10^{3}$	$9.14x10^{-3}$ $2.63x10^{4}$	$3.48x10^{-7}$ 4.01	0.999

D.6.2 Relative error

a. The error of each predicted to corresponding measured value was calculated as a fraction of each measured value. i.e. the deviation at a particular point or *point error*. This was divided by the standard deviation of the measured population and converted to a percentile scale to give the *relative error* for each part of the model. If the *point error* exceeded 100% of the standard deviation of the measured population, then there was a significant difference between the two populations at that point. In general, the closer the mean relative error value to zero, the closer the model has estimated the mean of the measured population, while the standard deviation of the relative error (SD*re*) indicates the predicted population's range of error.

A potential weakness in this method was that when a variable demonstrated a very large numerical range over the course of an experiment, the relative error would tend to be larger in the small range and smaller in the large range of the raw data. i.e. the relative error converged to zero as the measurement range increased. This effect was only noticeable in the liver enzymes in which there were extremely large measurement ranges (from 0-7000 IU/l). These enzymes have no prognostic value in ALF and for this reason the effect could have been ignored. However, for the sake of thoroughness,

b. A quantitative index for comparison was calculated by multiplying the measurement range with the standard deviation of the relative error (SD*re*) (table D.6.2.1). The larger the returned value, the larger the prediction *error region*. This represents a method of indicating the *average relative accuracy* of the various parts of the model. What must be borne in mind, especially in the biochemical variables, is that although the deviation in the relative error may have been small, the measured value range was often very large. In practice the point errors may still have been large.

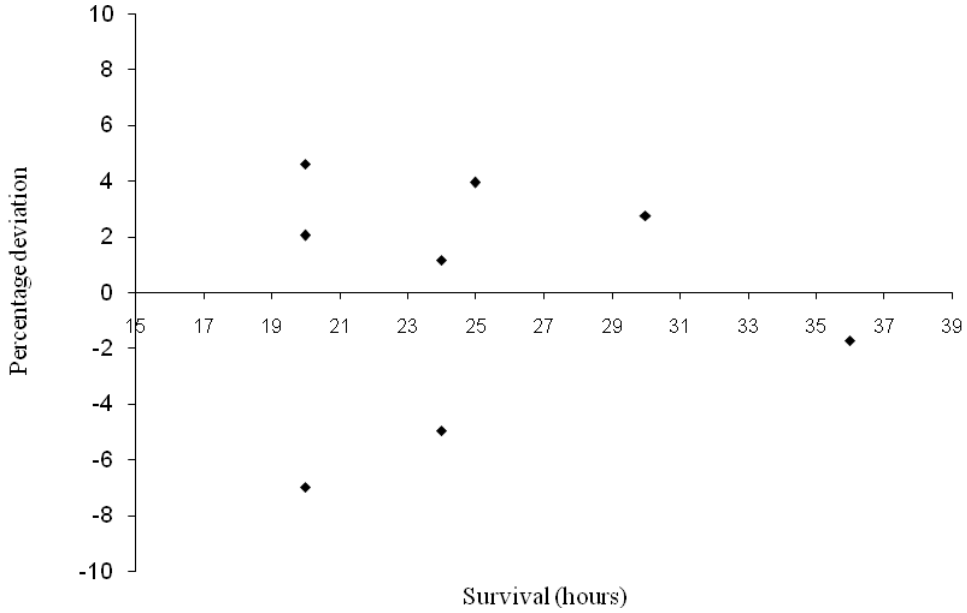


Figure D.6.2.1 Relative prediction error for the PI (T<0)

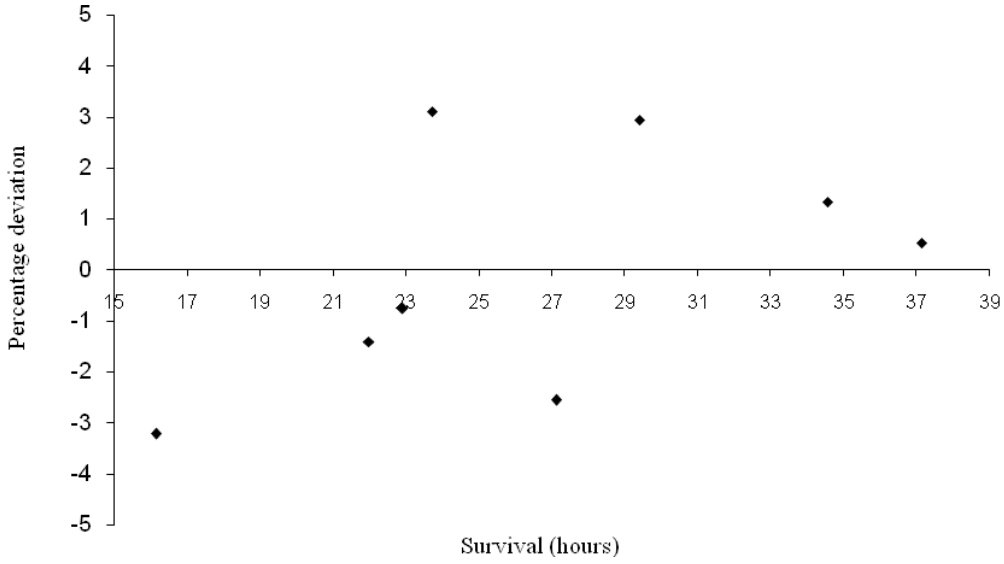


Figure D.6.2.2 Relative error for PI (T>0)

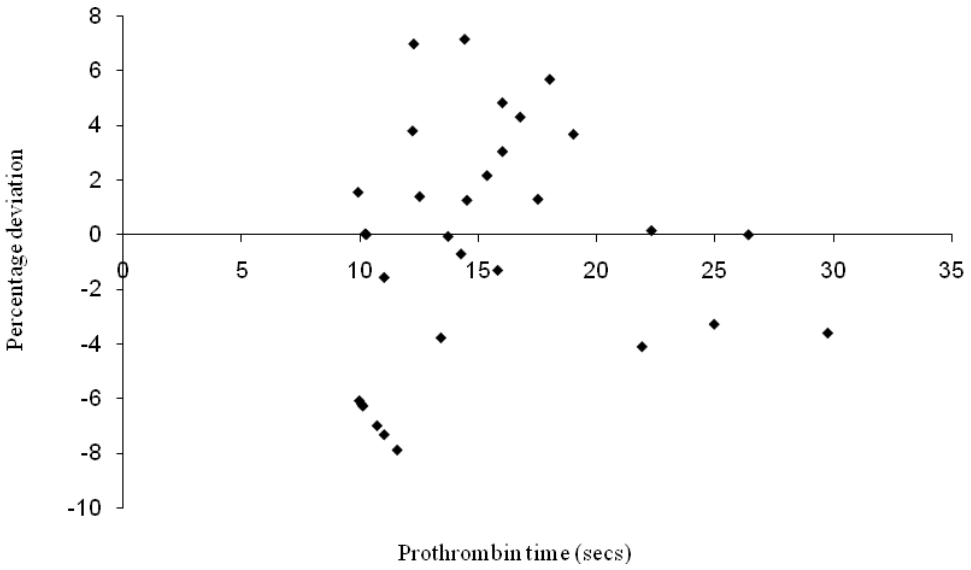


Figure D.6.2.3 Relative error for the BI (Prothrombin time)

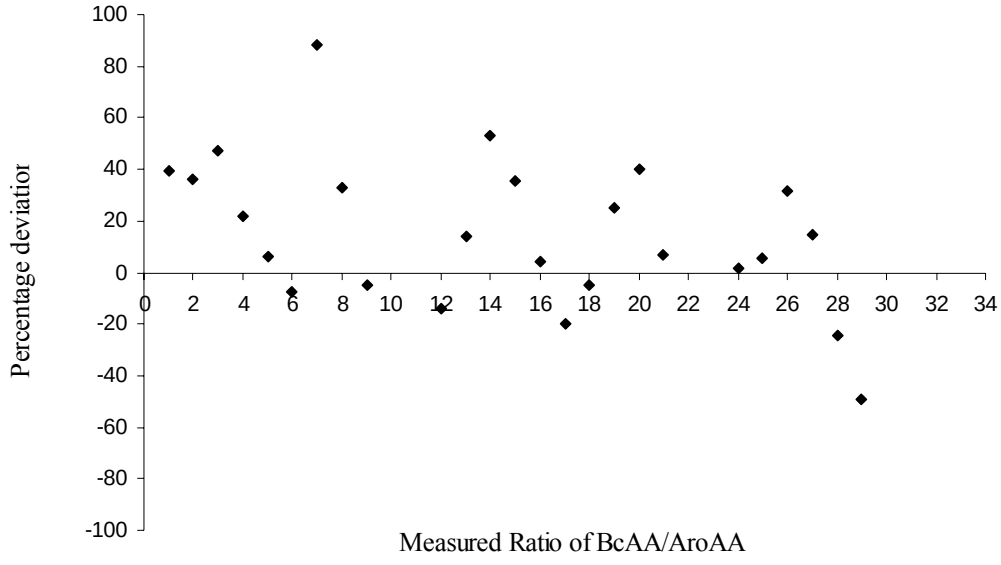


Figure D.6.2.4 Relative error for the BI [BcAA/AroAA]

Table D.6.2.1 Comparative accuracy of the PI and BI models (using 'training' data)

Model	**Measured values**			**Accuracy Index**		
Index	Max	Min	Range	Mean	SD*re*	Range*SD*re*
Prognosis T<0	36	20	16	0.14	4.24	67
Prognosis T>0	36	20	16	0.001	2.39	38
BcAA/AroAA	3.75	1.19	2.55	1.67	29.00	48.43
BilirubinTOT	30.0	1.0	29.0	-0.91	7.28	211.2
Fibrinogen	2.97	0.50	2.47	2.12	27.84	68.76
PT	29.75	9.90	19.85	-0.41	4.31	85.49
AntiThrombin	112.89	14.00	98.89	0.04	1.21	119.44
Factor II	59.65	7.61	52.04	-0.03	2.46	127.87
Factor VII	74.95	6.19	68.76	-0.08	3.28	225.54
Factor X	104.96	1.00	103.96	0.98	3.94	409.93
ALP	1206.0	57.0	1149.0	-0.01	0.32	366.9
AST	6607.0	23.0	6584.0	0.01	0.07	441.1
LD	6161.0	155.0	6006.0	-0.001	0.05	301.2
ALT	377.0	23.0	354.0	0.15	1.15	405.2
Creatinine	284.8	11.5	273.3	0.15	1.72	468.8
Urea	4.6	1.6	3.2	67.71	173.81	556.2
Glutamine	445.0	76.8	368.2	0.63	0.94	344.2

Notes:
1. *SD*re* = Standard deviation of the relative error
2. The closer the mean prediction error to 0, the closer the mean measured value has been estimated.
3. The smaller the Range*SD*re* the smaller the *error region* and the more accurate the predictions.

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