6. MATHEMATICAL MODELING STUDIES

Overview

The following section presents two mathematical modeling studies that have been developed with BAL system design-optimization and improvement in mind. These models are abstractions of a numerical rather than biological nature and differ in this respect to the preceding sections. The models presented are to some extent functionally complimentary and one logically leads to the next.

The first study details a pharmacokinetic compartment 'mass balance' model of the BAL-patient system. The value of models of this type include determining the effect of system parameters (such as for example the minimal hepatocyte mass, blood exchange rates, internal circulation rates and the system volume) on the accumulation of endogenous toxin by the patient. The minimal requirements for an effective BAL design are consequently provided by the findings of this model. A discussion follows regarding the limitations of models of this type, including measurement variations and their units, the type of variables that may be employed and the off-line nature of the predictions.

The second study describes a data-driven statistical model of ALF. This model is defined following conceptual UML modeling of the disease syndrome and the analysis of clinical data generated in the ischemic animal model experiments as described in the *in vivo* section preceding this one. This model functions as an on-line prognosis indicator and is designed for patient monitoring and parallel use during BAL clinical treatments. The findings are presented along with comparison to BAL treatment data on which the model had not previously been trained. A discussion details the merits and limitations of presently existing ALF prognosis systems, the conceptual and mathematical methods employed in this study and the practicalities of implementing the proposed on-line system.

The thoughts and recommendations that follow reviews the value/success of the above models then discusses the requirement of refining existing prognostic systems, additional mathematical experiments conducted on the above data, a means of combining the models of the above two studies and additional practical considerations in implementing on-line bioprocess monitoring systems.

6.1 A pharmacokinetic compartment model of the UP-CSIR BALSS

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Manuscript in preparation.

6.1.1 Introduction

Pharmacokinetics has historically been employed in quantitatively evaluating the functions of absorption, production and elimination of drugs or metabolites by BALSS bioreactors [250,251]. Pharmacokinetic compartment models employ the principle of the conservation of mass within a closed system. Ordinary differential equations (ODEs) are used to describe the molar concentration changes or flows of the substance/s between the compartments of the modeled system.

The value of such models includes comparing endogenous toxin production rates by the patient with bioreactor clearance rates and the effect on the above of system parameters such as circulation rates and reservoir volumes. Indications of the requirements of an effective BALSS design are also provided, such as the minimal hepatocyte mass in the bioreactor and the system's blood exchange and internal circulation rates.

The *in-principle* results of models of this type have been well elucidated [251-254]; however, few authors have provided actual quantitative results in prior studies [255-257]. This may be due to: Difficulties in obtaining reliable and comparable *in vitro* data on drug or metabolite clearance/production rates. The fact that the ideal functions of a BALSS bioreactor are incompletely defined [1,68], and possibly due to unappealing predictions of efficacy from the models themselves. For example, *in-principle* models have demonstrated that a BALS's clearance/production ability is directly proportional, to a first order, to the amount of cells in the bioreactor. Since the majority of researchers have used only between 2 and 5 % of the total mass of hepatocytes in a liver, the

expected metabolic capacity of the bioreactor cannot significantly exceed that range [251]. The limiting effects of the cell isolation procedures on hepatocyte phenotype and function can also not be ignored.

The results provided in this study are instructive in indicating innate limitations in BALSS clinical efficacy imposed by design factors. The findings of other authors and limitations in pharmacokinetic modeling methods are also examined.

6.1.2 Materials and methods

A simplified compartmental pharmacokinetic model of the UP-CSIR BALSS was built in Mathcad[1]. The diagram below (figure 6.1.1) is a simplified compartmental representation of the BALSS, with each part of the circuit numbered (compare with figure 2.5). For the model's derivation, nomenclature, units and parameters used, please refer to Appendix C.

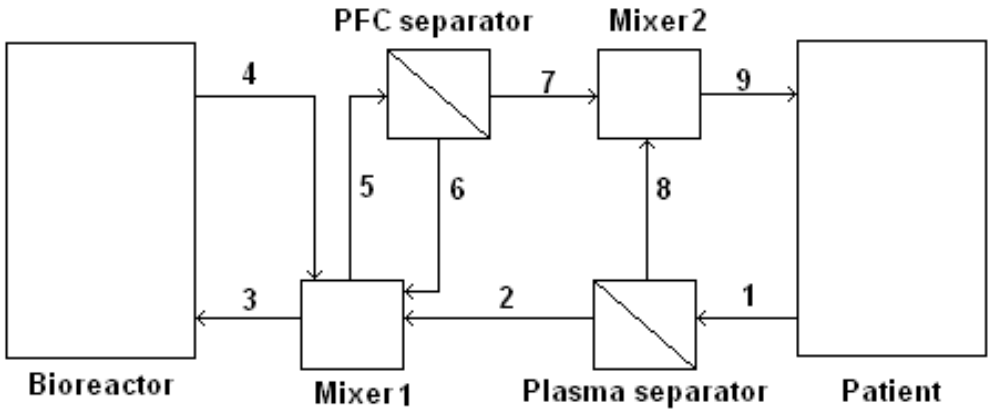

Figure 6.1.1 Compartmental diagram of the BALSS system connected to a patient

6.1.3 Data

The type of input data for such models is limited to drugs or metabolites that are alternately cleared and/or produced by the bioreactor and patient respectively. This

[1] The conceptual model was originated by Dr S Moolman of the M&Mtek [CSIR] and the author. All *in vitro* data was generated by the author. The numerical Mathcad modeling was performed by Dr M Shatalov of Materials and Manufacturing [CSIR]. Model derivation is in Appendix C.

allows the quantification of the conserved molar flow of the input variable between the compartments of the system, in terms of volume and concentration units per time. Since few drugs/metabolites other than arterial ammonia (which is endogenously produced in ALF) fit this characteristic, ammonia data was collected in two sets of experiments: Specifically, hepatocyte bioreactor clearance values measured in *in vitro* configurations of the BALSS (section 4.2) and *in vivo* endogenous ammonia production rates in animals that had surgically-induced ischemic ALF as previously described (section 5.2).
Additional reasons for selecting ammonia data included:

1. The existence of a readily accessible clinical laboratory measurement method with accuracy on the µmol/l level.
2. The fact that ammonia is the predominant substrate for the production of urea by hepatocytes, and is therefore a measure of liver function.
3. Several research groups have reported ammonia clearance values for porcine primary hepatocytes.
4. The overproduction of ammonia in ALF is considered to be an important cause of HE, and thus prognosis for survival.
5. It was approximately linearly endogenously produced in the ischemic ALF model previously described (section 5.2).
6. The possibility that the output of the pharmacokinetic model could be used as the input of another model subsequently investigated (section 6.2 below)

6.1.4 Results

The effect of adjusting particular system parameters on the model's outputs are as follows (figures 6.1.2-5):

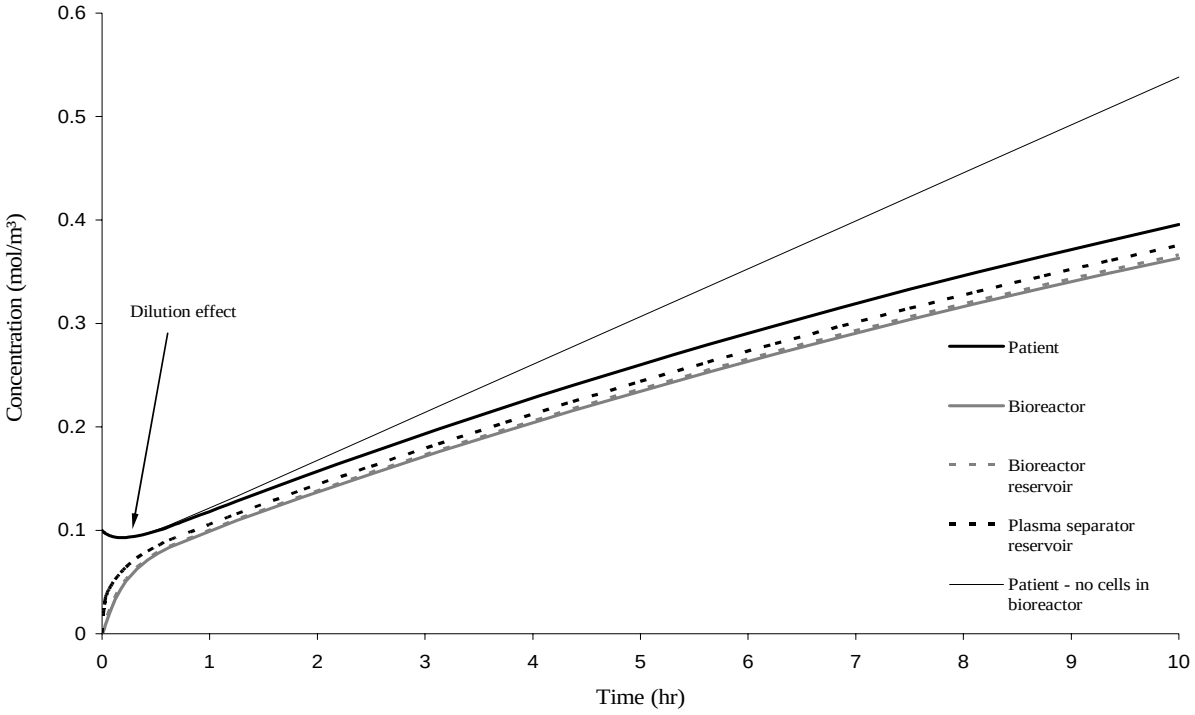

Figure 6.1.2 BALSS sub-circulation concentration profiles for ammonia.
Parameters: 1.5 x 10^{10} cells, V_{max} = 2.2 x 10^{-17} mol/s/cell, r_p = 4.2 x 10^{-8} mol/s, 120 ml/min blood exchange rate

1. The effect of including/excluding cells in a bioreactor (figure 6.1.2). Ammonia concentrations in the various parts of the BALS system can be seen to follow similar *rising* trends regardless of the presence of a cell-loaded bioreactor or not. However, the presence of cells (in this case 1.5 x 10^{10} or approximately 10 % of liver hepatocyte mass) will function to decrease the rate of accumulation. i.e. ammonia will always accumulate in an ischemic model of ALF due to the presence of a non-functional and progressively necrotizing liver, but treating with a cell loaded bioreactor will decrease the rate. A dilution effect in the system, caused by the saline priming volume of the BALSS, is observable in the first 30 minutes of the treatment. This effect has been indicated as a possible cause of improvements in patient survival in human clinical tests [251]. However, this is a limited perspective in that prognosis in ALF is multifactorial. i.e. it is not limited to blood toxin accumulation or artificial ALF treatment methods would historically have demonstrated improvements in survival.

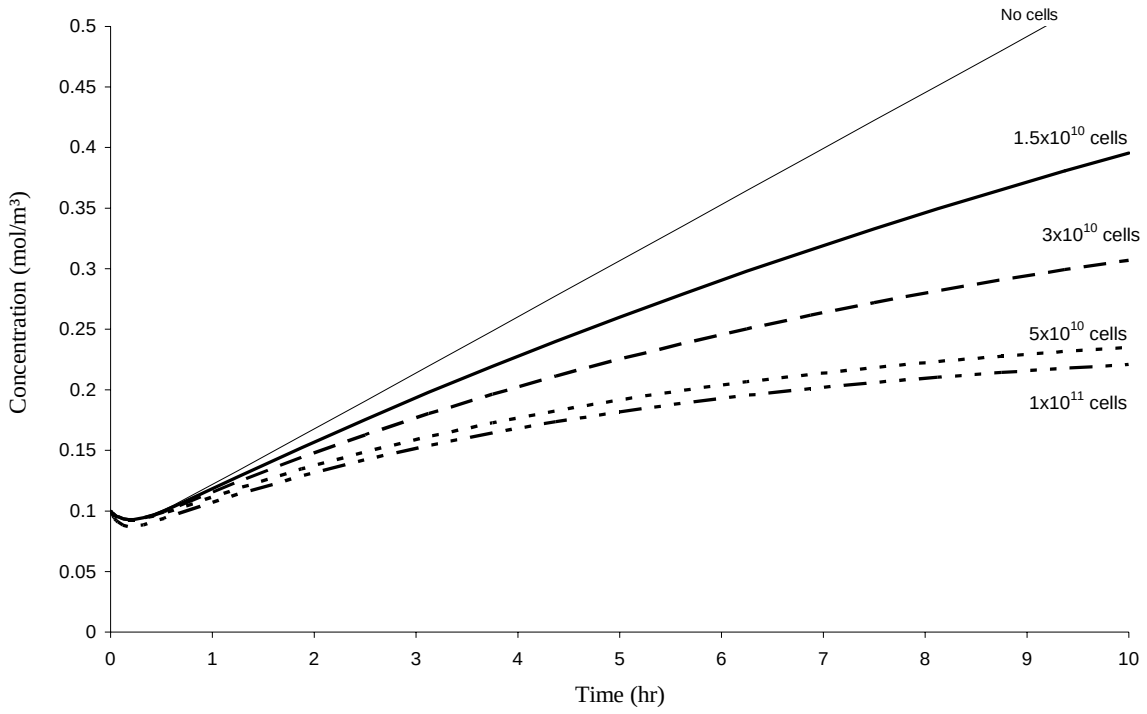

Figure 6.1.3 The influence of bioreactor cell loading on ammonia accumulation
Parameters: V_{max} = 2.2 x 10^{-17} mol/s/cell, r_p = 4.2 x 10^{-8} mol/s 120 ml/min blood exchange rate

2. The effect of cell numbers in the bioreactor (figure 6.1.3). As cell numbers increase, the equilibrium concentration tends to a limit that is higher than the starting (or normal) blood ammonia concentration. A human adult liver contains approximately 1- 2 x 10^{11} hepatocytes. Thus, adding cells even to the point of replacing the entire hepatocyte mass is insufficient to replace a liver's function. This is due to the fact that a BAL is an extracorporeal circulation system with a lower blood exchange/perfusion rate than an *in vivo* liver. Since the bioreactor is intrinsically unable to clear all of the ammonia, the above observation validates the use of adding an additional artificial detoxification device in the BAL circuit. Of interest, an increase in the bioreactor's cellular ability to metabolize ammonia, i.e. the V_{max}, would also result in a decrease in equilibrium blood ammonia levels. This demonstrates the desirability of improving cell functionality.

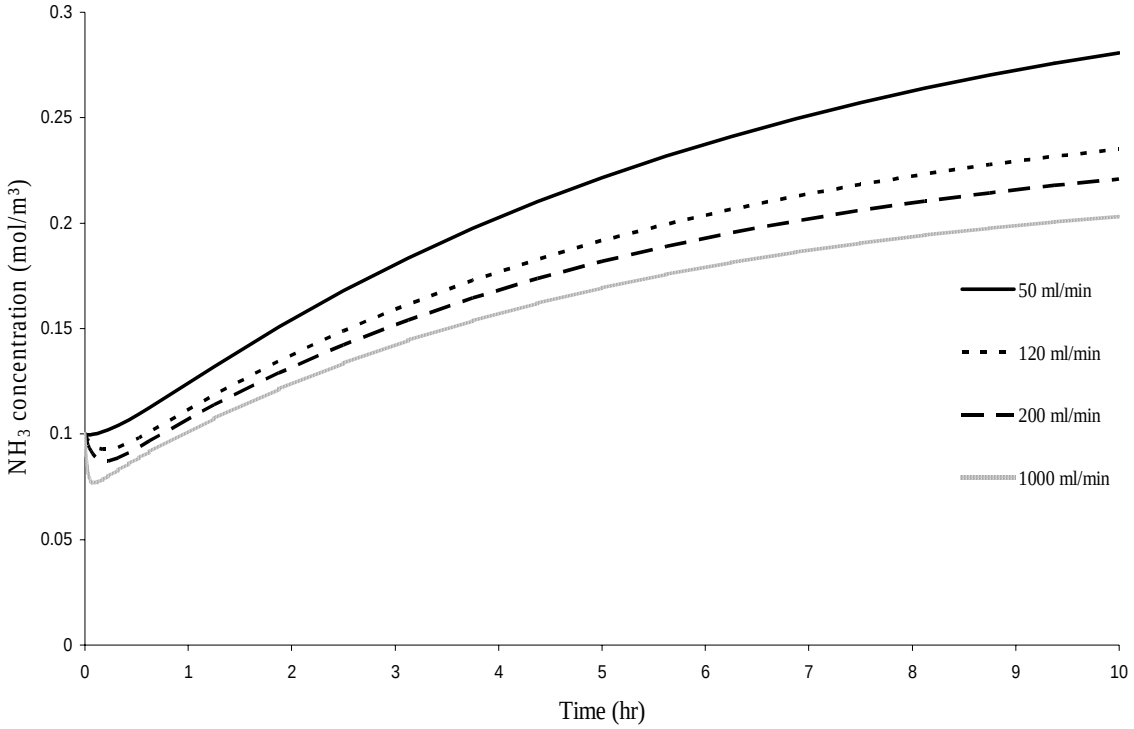

Figure 6.1.4 Influence of blood exchange rate on ammonia accumulation in the patient Parameters: 5 x 10^{10} cells, V_{max} = 2.2 x 10^{-17} mol/s/cell, r_p = 4.2 x 10-8 mo/s

3. The effect of the patient's blood exchange rate with the BALSS (figure 6.1.4). Lower exchange rates between the patient and the BALSS lead to higher ammonia concentrations in the patient. As the exchange rate increases the dilution effect becomes more pronounced, but compresses into progressively shorter time periods. A limit in ammonia concentration is reached at very high exchange rates, e.g. above 1000 ml/min. However, rates above approximately 200 ml/min are difficult to reach, due to e.g. the plasma separator pressure rating. Thus, it is clear that BAL toxin clearance is inherently limited in terms of replacing liver function.

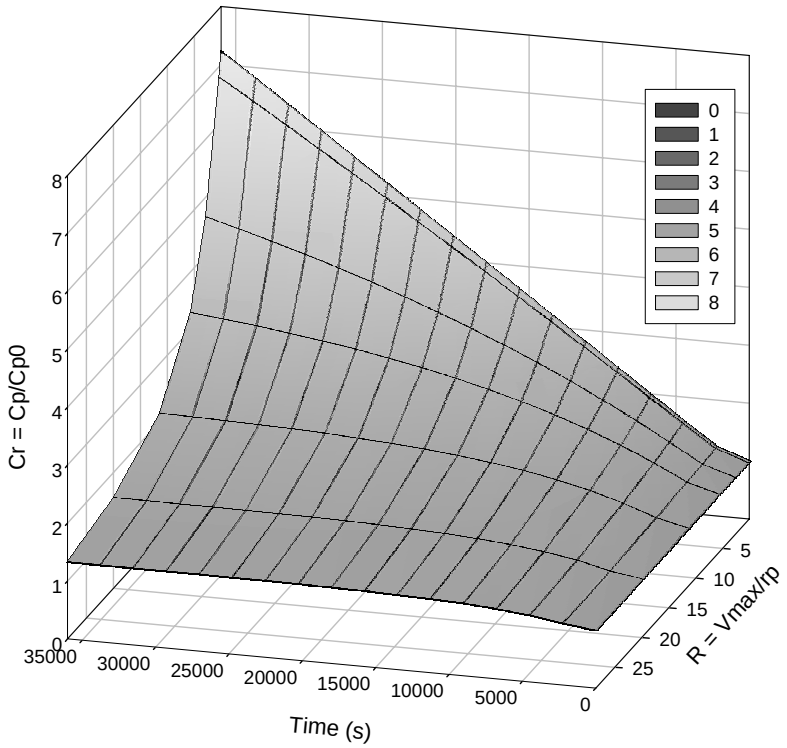

Figure 6.1.5 The influence of the clearance to production ratio on blood ammonia concentration. Graph key: Cr = ratio of actual to initial (normal) concentration, R = ratio of maximum to actual generation rates

4. The effect of the clearance rate (indirectly 'cell functionality') (figure 6.1.5). At low clearance rates ammonia concentration continues to rise and even after 10 hours of treatment, an equilibrium concentration (between actual and starting) may not be reached. At high ratios of clearance to production, an equilibrium may be reached within as little as an hour and at a low starting concentrations. This illustrates the importance of examining actual cell numbers and cell functionality when designing a BALSS. Interestingly, the initial ammonia concentration (C_{p0}) influences the shape of the surface, but not the ultimate equilibrium value.

6.1.5 Discussion

Hepatocytes are the cells accounting for the majority of liver toxin clearance functions and the synthesis of hepatotrophic (liver regenerative) substances. However, artificial blood toxin clearance devices, such as for example activated charcoal filters, are known to be better at clearing toxins than most bioreactors [251]. It is presumably for these reasons that cell-based biological systems have shown improvements in survival (of treated animals), while purely artificial liver support technologies have not (despite the extensive use of the latter in human treatments).

Iwata *et al* (2004) [251] clearly elucidated the results of BAL modeling efforts. Our simulations confirmed these findings. In summary,

1. The clearance value of a BALSS is proportional, to a first approximation, to the number of hepatocytes in the system. i.e. the blood toxin concentration of a patient treated with a device containing a small number of hepatocytes will stabilize at a concentration that is several times higher than the normal (starting) concentration, even after long-term assistance.
2. If an adult is treated with a BAL containing, say, 10 % the hepatocyte mass of an innate liver, the patient's blood toxin concentration will stabilize at a concentration that is approximately 10 times higher than that of the normal (starting) concentration, even after long-term assistance. There is an approximately inverse linear relationship between the hepatocyte mass and the eventual toxin concentration. A liver weighs approximately 1500 g, of which 80 % or 1200 g are hepatocytes. The majority of current BAL bioreactors employ in the region of 50-100 g of hepatocytes.
3. The clearance value of a bioreactor cannot exceed the perfusate flow rate, thus, the plasma exchange rate provides the upper limit for the clearance of toxins. However, the clearance values of most artificial detoxification modules is between 10 and 15 times greater than that of existing bioreactors, thus, the use of these modules is attractive in terms of blood toxin removal in liver support. No existing BAL design can eliminate toxins, such as ammonia, as rapidly from the

systemic circulation as an adult liver. However, should an artificial clearance device be added to a BAL system care should be taken to ensure that filtration does not exclude desirable low molecular weight substances, such as growth factors, from entering the patient and providing regenerative benefits.

4. Since a bioreactor contains, say, only 10 % the hepatocytes of a normal liver, it can only produce a maximum of 10 % of the plasma proteins of a healthy person, even if the patient is treated for an extended period. Thus, supporting a patient by means of plasma exchange is likely to be more efficient than with a BAL alone. However, large amounts of plasma are expensive and animal sources for the treatment of human patients are unacceptable due to concerns regarding xenogenicity. Difficulties also reside in isolating and culturing the very large quantities of metabolically functional cells required.
5. Only a BALSS that has an exchange rate that is capable of matching the blood inflow rate of an innate liver will match the functionality of a normal liver. The blood inflow rate of an adult *in vivo* liver is in the region of 1500 ml/min, while blood exchange rates to a BALSS lie in the 100 - 300 ml/min region. Thus, in terms of treatment, only OLT is able to meet this objective, which may explain why existing systems that have undergone human clinical trials have not shown significant survival benefits. It also explains why the benefit of BAL treatments is expressed as a ‘bridging’ therapy to OLT.

However, there are limitations to pharmacokinetic modeling methods:

1. Measurement variations. As was evidenced in section 4.2, the pharmacokinetic clearance/production values reported for porcine hepatocytes are exceptionally variable. Accurately simulating clearance or production ability of a bioreactor may consequently be difficult. A basic review indicates an excess of a 10 000-fold divergence across reported values (table 4.2.1 and figure 6.1.6).

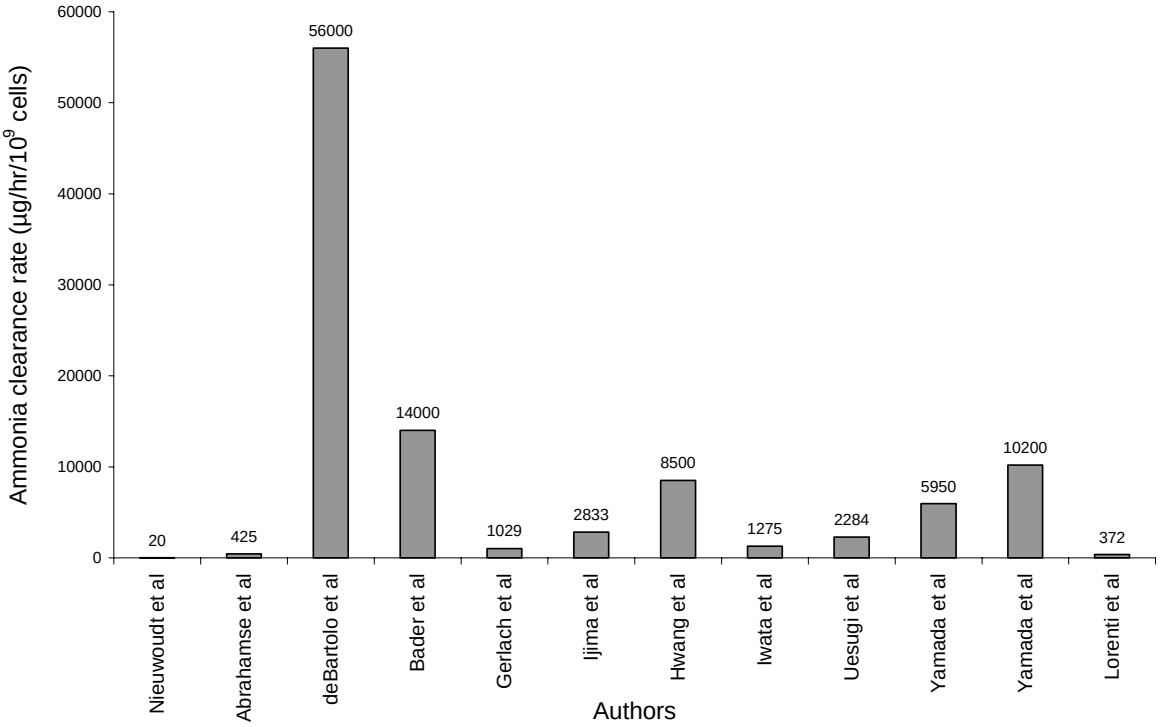

Figure 6.1.6 Variation in reported values of ammonia clearance

2. Measurement units. The means by which clearance values are reported may prevent the direct comparison of values. Data is often reported in units of µg/hr/10^9 cells, which is normally calculated directly from the slope of, e.g. the graph of ammonia, concentration against time and the number of hepatocytes in the system. However, this does not indicate the inherent ability of the cells in the system to clear ammonia. Other factors may affect the results, for example, the starting concentration of ammonia in the system and the ratio of the number of cells to the reactor volume. It would seem highly unlikely that primary porcine hepatocytes would exhibit 10 000 fold variations in ammonia clearance rates based on relatively small changes in reactor size, flow rates or scaffold materials. In an attempt to enable more accurate comparisons between reported values we calculated the parameters Vmax and Km from the raw data (as described in Appendix C). In this case a reduced Vmax (Vrmax) was used, taking into account the cell loading per reactor volume ratio.

3. Measurable variety of liver functions. Pharmacokinetic modeling is limited to only the clearance of toxins and/or synthesis of plasma proteins or metabolites. These must also be readily measurable. While these functions are necessary, there are also other important liver functions in the context of ALF, for example, regeneration. In this process numerous cytokines, chemokines and growth factors participate in complex cell-signaling cascades that initiate and control each step. Hepatocyte growth factor (HGF), epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α) are viewed as the primary stimuli, which are potently mitogenic, but require that hepatocytes first be primed by other soluble factors, including tumour necrosis factor (TNF) and interleukin-6 (IL-6) [258,259]. In ALF, the concentrations of HGF and EGF in the plasma may rise to a maximum of only10 ng/ml, yet this is sufficient to initiate regeneration. This may be viewed as a complex non-linear *on-off* switch, rather than a linear response to the extent of liver damage.
4. *Off-line, after-the-fact*, single metabolite per simulation methodology. Although pharmacokinetic models are a necessary and desirable part of finalizing BAL design they provide predictions regarding the clearance or production of only single substances at a time and only after rates of production and or clearance have been measured in *in vitro* and *in vivo* experiments. However, once this data is available and if one assumes they will remain the same in a treatment, the model can be adapted for an *on-line* application in which, say ammonia is measured on a constant basis.

In summary, while compartmental pharmacokinetic models provide information of value in especially the design of a BALSS, they are limited to describing only the clearance and or production of individual biochemicals. ALF is a complex; multisystemic process that is incompletely understood and consequently the full range of BAL functions are not precisely defined. Other analysis and modeling methods are additionally required.

6.2 Developing an *on-line* predictive clinical monitoring system for acute liver failure patients

Nieuwoudt M, Bond R, van der Merwe SW, Cilliers P.

Manuscript in preparation.

6.2.1 Introduction

A variety of prognostic criteria have historically been proposed for the early identification of acute liver failure (ALF) patients that are likely to die and therefore require orthotopic liver transplantation (OLT), the only treatment of proven benefit. These criteria have, without exception, been defined based on the multivariate analysis of clinical variables measured on patient admission to the clinic [52-56,239-241,260-269]. Owing to the multiplicity of etiologies and pathogenesis in ALF [24-26,72] a large range of variables with prognostic value have been identified, and studies of new/additional variables have subsequently continued [239-241,267,268].

The rate at which a clinician is able to update a patient's prognostic score is based on the regularity at which the involved clinical variables are sampled. For example, blood indices are typically measured 12 or 24 hourly. However, the progress of ALF is rapid with a high mortality rate within 5-10 days following diagnosis. Thus, a pressing need remains for prognostic criteria which are able to provide early and repetitively accurate estimates of prognosis in ALF. Clinical microdialysis (www.microdialysis.se**)** has recently become an attractive addition to the ICU in that bedside systems are able to provide *on-line* indications of physiologically interesting 'biomarkers' using sterile probes and low volume sampling techniques [270,271]. Thus, a possibility exists to combine high-frequency systemic and biochemical monitoring in the ICU with one or more prognostic models. This would be a 'dynamic' prognostic system which would be of value to critically ill ALF patients in whom rapid clinical decision-making is necessary. Predicting outcomes in (expensive) animal experiments may also allow earlier terminations and thus benefit researchers investigating, for example, bio-artificial liver (BALSS) devices.

This study proposes a variety of modeling and hardware implementation methods for the development of a combined *on-line* biochemical and prognosis monitoring system. The underlying models include a conceptual part defined using the Unified Modeling Language (UML) followed by a numerical part employing a multivariate statistical approach. The UML is a graphical systems engineering tool that specifies systems structure (composition) and behaviour (function). It is often used to define software architectures [272-278] and has been proposed as an ideal method for integrating biological data into *in silico* models and meta-models in hierarchical levels of complexity [276-278]. Both the UML and subsequent statistical models aim to represent the multi-systemic pathogenesis of ALF. Technically, the resulting model is a *state estimator* [11,279-283] of ALF and is *data-driven* [284] in that it is based on trends in raw clinical data collected in irreversible (i.e. resulting in death) surgically-induced ALF experiments in pigs, as previously described by our group [213]. It is understood that the proposed model/s are currently relevant to the animal circumstance. Future efforts combining additional animal and human data may subsequently facilitate the implementation of an *on-line* system in the human clinical scenario.

6.2.2 Methods

6.2.2.1 Data processing

The data of a large number of systemic and biochemical variables was collected in the course of experiments aimed at standardizing an ischemic surgical model of ALF in pigs as previously described [213] (tables 5.2.1,2). Of this, 8 cases were selected as ideally representative of future experiments and were used as the training subset in defining the numerical models described below. Thereafter, the *un-trained* data of 8 animals, which were involved in subsequent clinical evaluations of the UP-CSIR BALSS, was used in testing the accuracy of the model. Excel 2003 was used as the spreadsheet for all data, including linearity testing, a macro for Tornado sensitivity diagrams [285], Monte Carlo analysis [286,287] using the random number generator facility and analysis of variance (ANOVA-single factor without replication, 0.05 confidence level). Statistix 8 (Analytical

software, Tallahassee, FL, USA) was used to calculate parametric and non-parametric correlation coefficients and Shapiro-Wilks normality tests.

6.2.2.2 Conceptual (system) modeling

The UML diagrams were made using an IBM-Rational Rose UML modeling tool. The class diagram (figure 6.2.1) provides a hypothetical structural framework for the data attributes of the objects (organs-systems) of the ALF patient-system as a whole. Each class contains the measurable attributes (variables) for the *minimally* involved organs or organ-systems. The associations (arrows) between classes indicate the 'inheritance' of each sub-system, i.e. the composition of each class. Some associations are necessarily bi-directional in that the components (organs) of a physiological system are inter-related. Not all of the variables listed in the class diagram were measured in the pig experiments. The list of attributes (table 6.2.1) is composed of those variables identified in a range of studies [24-26] (and by speakers at the European association for the study of the liver (EASL), Acute Liver Failure congress of 2007 [288,289]) as useful in indicating particular stages in the pathogenesis of ALF in a patient. Naturally, more data attributes may be added or subtracted as knowledge of their prognostic utility develops.

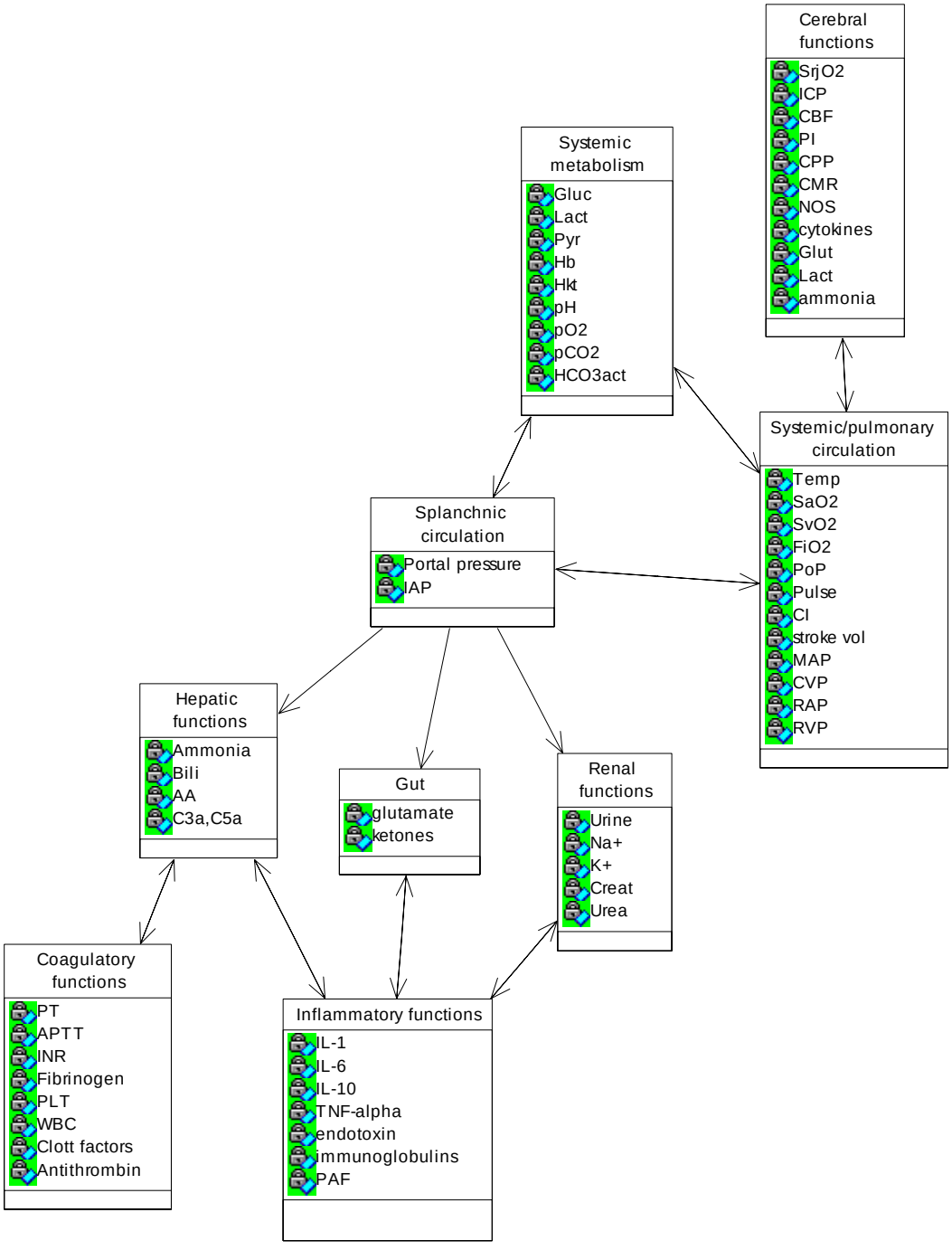

Figure 6.2.1 Class diagram for data attributes (clinical variables) of the patient-ALF-system. Each class represents an organ-system where the measured variables provide information of prognostic value in ALF. Not all the variables were measured in the animal experiments. These may be incorporated into subsequent numerical modeling instances.

Table 6.2.1 Attributes (clinical variables) for the class diagram.
The highlighted variables were not measured in the animal experiments (section 5.2).

Class	Attribute (variable)	Description	Units	Behaviour over time
Systemic metabolism	Gluc	blood glucose	mmol/L	homeostatic
	Lact	blood lactate	mmol/L	increasing
	Pyr	blood pyruvate	mmol/L	homeostatic
	Hb	blood hemoglobin	g/dL/hr	decreasing
	Hkt	blood hematokrit	%	decreasing
	pH	blood pH	pH	homeostatic
	pO2	partial pressure of O_2	mmHg	homeostatic
	pCO2	partial pressure of CO_2	mmHg	homeostatic
	HCO3act	blood activated bicarbonate	mmol/L	homeostatic
Systemic/ pulmonary circulation	Temp	temperature	^oC	increasing
	SaO_2, SvO_2	central arterial and venous O_2 saturation	ml/dl	decreasing
	FiO_2	% inspiratory O_2 concentration	%	
	PoP	pulmonary occlusion pressure	mmHg	
	Pulse	pulse rate	beats/min	homeostatic
	CI	cardiac index	ratio	
	Stroke vol	left ventricular stroke volume	ml	
	MAP	mean arterial pressure.	mmHg	decreasing
	CVP	central venous pressure	mmHg	
	RAP	right atrial pressure	mmHg	
	RVP	right ventricular pressure	mmHg	
Cerebral Functions	$SrjO_2$	reverse jugular venous O_2 saturation	ml/dl	decreasing
	ICP	intracranial pressure	mmHg	increasing
	CBF	cerebral blood flow velocity	ml/min	
	PI	Pulsatile index	ratio	
	CPP	cerebral perfusion pressure	mmHg	
	CMR	cerebral metabolic rate	µmol/g/min	
	NOS	nitric oxide synthase	µmol/L	
	cytokines	see IL and TNF below	µmol/L	
	Glut	glutamine	µmol/L	
	Lact	lactate	mmol/L	
	ammonia	ammonia	µmol/L	
Splanchnic circulation	portal pressure	hepato-portal pressure	mmHg	
	IAP	intra abdominal pressure	mmHg	
Hepatic Functions	ammonia	ammonia in blood	µmol/L	increasing
	bilirubin	bilirubin in blood	µmol/L	increasing
	amino acids	ratio of branched-chains to aromatic	ratio	decreasing
	complement	C3-a and C5-a in blood	µmol/L	
Gut	glutamate	glutamate in blood	µmol/L	
	ketones	ketones in blood	µmol/L	
Renal Functions	Urine	urine volume	mL	constant
	Na+	change of [sodium]	mmol/L	decreasing
	K+	rate of change of [potassium]	mmol/L	increasing
	Creat	rate of change of [creatinine]	µmol/L	increasing
	Urea	rate of change of [urea]	mmol/L	decreasing

Class	Attribute (variable)	Description	Units	Behaviour over time
Coagulatory Functions	PT	rate of change of prothrombin time	secs	increasing
	APTT	rate of change of activated partial thromboplastin time	secs	increasing
	Fibrinogen	rate of change of [Fibrinogen]	g/L	decreasing
	PLT	rate of change of platelet count	10^9/L	decreasing
	WBC	rate of change of white blood cell count	10^9/L	increasing
	Clott Factors II	percentage of normal clotting factors II, V, VII, IX, X in blood	%	decreasing
	AntiThrombin	rate of change of percentage of normal anti thrombin in blood	%	decreasing
Inflammatory functions	IL-1,6,10,	interleukins 1,6,10	µmol/L	
	TNF-alpha	tumour necrosis factor-alpha	µmol/L	
	endotoxin	endotoxin	pmol/L	increasing
	immunoglobulins	immunoglobulins	µmol/L	
	PAF	platelet activating factor	µmol/L	

Note: In the surgical ischemic experiments all biochemicals/metabolites were measured in whole blood or plasma. Where behaviour over time is not included the trend must still be determined.

The purpose of a state transition diagram (figure 6.2.2) is to describe the behaviour of a system. In this case specifically the pathogenesis of ALF, albeit in a simplified way. In general the state of an object changes over time and transitions may be reversible. For example, in the human clinical scenario early ALF may revert to health (the initial state) owing to the innate regenerative ability of the liver. However, the degree of disease progression determines the likelihood of reversion (i.e. the further down in the diagram the worse the prognosis). In the ischemic surgical animal model, on the other hand, state transitions were irreversible in that death (the terminal state) was always the end-point. For this reason the resulting models are dependent on the particular data employed at this stage. Each state or sub-state may be defined based on a set of numerical values of the attributes (variables) of the organ-systems of the patient at any given time (table 6.2.2).

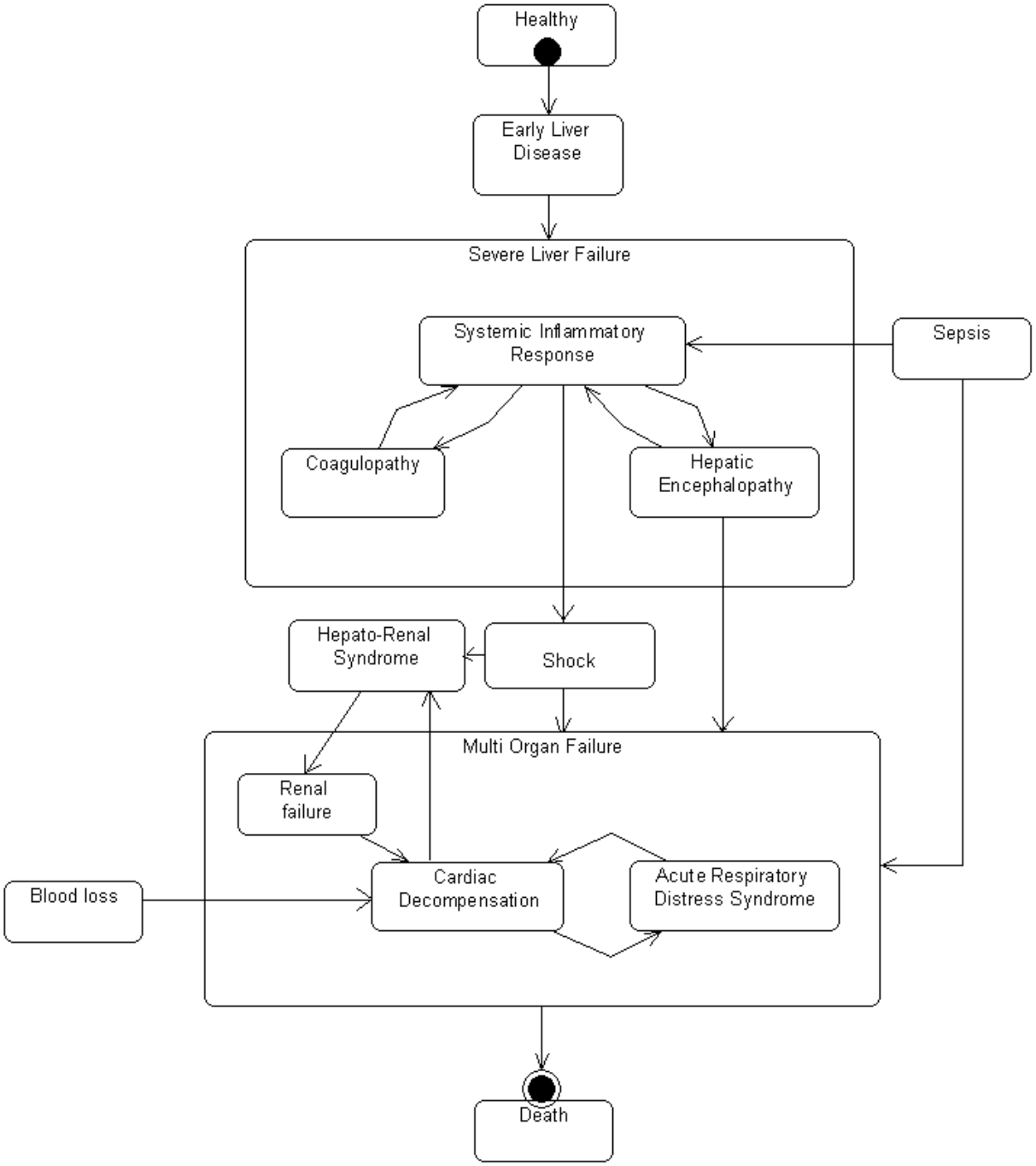

Figure 6.2.2 A system state transition diagram for the ALF-patient system in the ischemic surgical model. Defining state diagrams requires collaborative agreement between researchers and clinicians. Since ALF is only partially understood at present the above is understood as an iterative instance.

6.2.2.3 Numerical modeling

This was based on clinical variables that were found to have prognostic value in the porcine surgical model of ALF. Analysis of the data revealed that prognosis was intrinsically multivariate, with many variables simultaneously determining survival and none predominating [213].

Briefly, the 'raw data' was divided into two depending on the period in which it was acquired. In the initial surgical period (T < 0) in each experiment, the impact of the surgically-induced ALF intervention/s was measured in terms of predominantly systemic absolute-valued indices. In the latter intensive care (ICU) stage (T > 0), the progress of the disease was measured in terms of the rates of change (first derivatives) of predominantly biochemical variables. These rates were the mean gradients of the *best-fit* linear equations derived for each of the animals in each of the biochemical variables as a function of time and were considered the 'derived data'. Non-parametric (Spearman) correlation coefficients, for small data sets, were calculated between the durations of survival, the raw data from the surgical period and the derived data from the ICU respectively. Multivariate linear equations (internally weighted by their correlations) were then derived between the durations of survival (dependent variable), the surgical systemic data and the ICU biochemical trends (independent variables).

The method used was as follows: The dependent variable,

$$Y = \sum_{i=1}^{N} f(x_i) \cdot w_i \qquad (6.1)$$

where N = the total number of equations used to determine the dependent variable Y, $f(x_i)$, is the linear function between each independent variable x_i, and the dependent variable in question. The percentile weight, w_i is the following ratio:

w_i = (the specific weight between the $f(x_i)$ and Y) / (the sum of specific weights to N), (6.2)

where the 'specific weight' is the Spearman correlation coefficient between each independent and dependent variable. Thus, the sum of percentile weights would be equal to one. i.e.

$$\sum_{i=1}^{N} w_i = 1 \qquad (6.3)$$

The independent variables for the model were selected based on their satisfying the following criteria:

1. As high a correlation, or 'weight', with the dependent variable as possible (i.e. a clinical association of relevance in the animal ALF model).
2. As linear over time as possible, and
3. The existence of sensors and equipment for their o*n-line* measurement during experiments.

This method aimed to model the multi-systemic nature of ALF with a multivariate numerical approach. This *stabilized* the model's predictive ability by proportionally assigning weight to variables according to their prognostic value. This method is akin to estimating a multivariate non-linear function at any particular time by the Taylor expansion of its composing derivatives [286,290].

Two instances of the numerical model were created: a Prognostic indicator (PI) and a Biochemical Indicator (BI). These were designed to provide on-line predictions of, respectively, the duration of survival as a primary end-point measure of prognosis, and the numerical value of normally *off-line* biochemicals as secondary 'biomarker' end-points. i.e. the BI provides values for biochemicals normally requiring *off-line* laboratory analysis. Future iterations of such a model may have different end-points.

6.2.3 Results

For purposes of efficiency much of the model sensitivity and verification results have been moved to Appendix D. Only the results critical to demonstrating the accuracy of the model are presented below. For more information please refer (to Appendix D) as accordingly referenced.

6.2.3.1 Class associations

A simple physiological reasoning was initially applied to identify numerical associations of biological interest for potential quantification at a later stage. Correlational statistical analyses investigated the presence of numerical associations (arrows) within classes (i.e *internal* associations) and between classes (i.e. *external* associations) as shown in the class diagram (figure 6.2.1). A clearly multi-variate profile, as was found in section 5.2 was apparent. Since not all pairs of the associated variables are measurable *on-line*, these associations were of a theoretical interest at this point. Please refer to table D.1.1 in Appendix D.

6.2.3.2 Quantification of states

Thereafter, the states/sub-states of the state transition diagram (figure 6.2.2) using the attributes (variables) of the class diagram (figure 6.2.1) were numerically quantified (table 6.2.2). The value in quantifying these states is that an appropriately programmed *on line* monitoring system will be able to provide real time indications of changes in the patient's clinical state. This will facilitate more rapid clinical decision-making than has previously been possible.

Table 6.2.2 Examples of state and sub-state definitions (for reference see section 2.4)

State	Sub-state	Definition
Healthy		Mean values of all variables at start of experiments
Early liver disease		Values < 6 hrs after start
Severe liver failure		Values > 8 hrs after start
	Systemic Inflammatory Response (SIR)	2 or more of: a) fever (Temp > 38 ^{o}C) or hypothermia (Temp < 36 ^{o}C) b) tachypnoea (Respiration rate >24 breaths/min) c) tachycardia (Pulse > 90 beats/min) d) leukocytosis (>12000/µl), leukopenia (< 4000/µl), or >10% bands which may have a non-infectious aetiology
	Coagulopathy	International Normalized Ratio >1.5
	Hepatic Encephalopathy (HE)	I. Behaviour changes with minimal change in level of consciousness II. Gross disorientation , drowsiness, possible asterixis, inappropriate behaviour III. Marked confusion, incoherent speech, sleeping most of the time but arousable to vocal stimuli IV. Comatose, unresponsive to pain, decorticate or decerebrate posturing
Sepsis		Inflammatory response to a proven / suspected infectious etiology
Hypovolemic Shock		hypotension (arterial blood pressure < 90mmHg systolic, or 40mmHg less than normal) for > 1 hr despite adequate fluid resuscitation OR Need for vasopressors to maintain systolic blood pressure $\geq$ 90mmHg or MAP $\geq$ 70mmHg
Hepato-Renal Syndrome (HRS)		a. creatinine level > 220 µmol/L with portal hypertension b) no sustained improvement in renal function after volume expansion with isotonic saline solution Urine – a) volume < 500ml/d, b) Na < 10mEq/L, c) osmolality greater than plasma osmolality, d) red cell count < 50 per high power field, e) serum Na < 130mEq/L
Blood loss		Loss > 20% of total blood volume
Multi Organ Failure (MOF)		Dysfunction of more than 1 organ, requiring intervention to maintain homeostasis
	Renal failure	Renal urine output < 0,5ml/kg/hr for 1 hr despite adequate fluid resuscitation;
	Cardiac Decompensation	Adequate fluid resuscitation – pulmonary artery wedge pressure $\geq$ 12mmHg or central venous pressure $\geq$ 8mmHg
	Acute Respiratory Distress Syndrome	Respiratory $PaO_2/FIO_2 \leq 250$ or, if the lung is the only dysfunctional organ, ≤ 200
Death		Mean values at termination of experiments

Note:

1. The above are definitions arising from the human scenario; each state may be further numerically resolved in terms of limits in measured variables at particular time points.

6.2.3.3 The PI equations

Using the numerical method described above the PI was defined as follows:

Table 6.2.3 Model equations and weights for the PI

Independent Variable [x]	Time period	Survival Y = f(x) (hours)	Weight [Spearman correlation]	Percentile Weight Used	Survival data used for training
Body weight		y = -0.003x + 26	0.103	0.045	
Ischemic time		y = -1.16x + 43	0.805	0.354	
MAP_isch		y = 0.06x +22.2	0.012	0.005	
MAP_post		y = -0.083x + 32.2	0.417	0.183	
Pulse_isch	**T<0**	y = 0.072x + 14.3	0.196	0.086	
Pulse_post		y = 0.006x + 25	0.258	0.113	
Temp_post		y = -0.87x + 57.4	0.264	0.116	
Urine_oper		y = 0.021x + 23	0.218	0.096	
Range					20-36 hrs
Mean					*24.875 hrs
TOTAL			2.274	1.0	8 cases
rAmmonia		y = -0.2218x + 36.412	0.485	0.25	
ave_pH		y = -75.627x + 589.2	0.256	0.15	
rHb		y = -6.6337x + 32.459	0.400	0.20	
rHkt	**T>0**	y = 10.415x + 32.459	0.412	0.23	
rK+		y = -19.914x + 28.019	0.230	0.10	
rMAP		y = 5.5301x + 34.909	0.679	0.08	
Range					20-36 hrs
Mean					*26.625 hrs
TOTAL			2.463	1.0	8 cases

Notes:
1. The above variables were chosen as indicators of survival due to their availability for *on-line* measurement, linearity with time and clinical relevance in ALF. Subsequent modeling iterations may employ different variables.
2. In the initial period (T<0), the independent variables were chosen so as to indicate the impact of the surgical interventions. They were composed of absolute values, e.g. the suffix ' _isch' was the mean value of the particular variable during the ischemic clamping time. The suffix '_post' was the mean value after the ischemic time was over.
2. In the latter ICU period (T>0) the independent variables were for the rates at which the listed variables changed over time.

The underlying assumptions of the numerical model were tested as follows:

6.2.3.4 First-order assumptions

The predictive ability of the numerical model relies on the degree to which the independent variables of its composing first-order equations approximate either linear appreciation or depreciation over time. This assumption was investigated by determining the best-fit linear equation for each data set of all variables according to time. A mean R^2 value, the numerical square of the Pearson coefficient was calculated for all variables. The majority of all variables had R^2 values above 0.5 (i.e. Pearson coefficients > 0.7) indicating that variables did linearly change over time and justified the numerical design of the model.

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. For more details on these results refer to Appendix D.2.

6.2.3.5 The BI equations

The linearity of the variables in the T>0 part was intrinsic to defining the BI part of the model (tables 6.2.4,5). In this case, highly linear derived variables that were of prognostic value in ALF (and which could be monitored *on-line*) were chosen as independent variables. Firstly, best-fit linear equations were determined for the dependent variables according to time. Then first-order equations were derived that related the derivatives of the independent variables to the derivatives of the dependent variables.

The purpose was to determine the absolute value of a biochemical (that cannot be monitored *on-line*) at any particular time in an experiment using the rate at which a highly linear, independent variable (that can be monitored) was changing in real-time. The difference with the PI is that the absolute value of an *off-line* variable may be determined through the relationship of its first derivative with the derivative of another independent *on-line* variable.

Table 6.2.4 Variable candidates for the BI

Independent variables	**Ranked survival correlation**	**Linearity with time (R^2)**	**Dependant variables**	**Ranked survival correlation**	**Linearity with time (R^2)**
Ammonia	0.772	0.912	BcAA/AroAA	0.734	0.733
K+	0.492	0.569	Glutamine	0.248	0.608
Hb	0.599	0.872	Bilirubin	0.010	0.805
Hkt	0.535	0.808	Fibrinogen	0.327	0.880
			PT	0.370	0.922
			Antithrombin	0.120	0.878
			Factor II	0.208	0.882
			Factor VII	0.337	0.821
			Factor X.	0.347	0.785
			ALP	0.280	0.904
			AST	0.220	0.832
			LD	0.216	0.823
			ALT	0.010	0.781
			Creatinine	0.460	0.632
			Urea	0.301	0.508

Notes:1. The inclusion criteria for the independent variables were:
a. The variables had to be of interest in ALF.
b. The R^2 value, indicating linearity, should be ≥ 0.5.
c. The Spearman (ranked) correlation coefficient, indicating correlation with the duration of survival, must be ≥ 0.5. (K^+ was judged sufficiently close to this value for inclusion).
2. The inclusion criteria of the dependant variables were the same as for a. and b. above. However, as can be seen, the dependant variables demonstrated less correlation with survival than the independent variables. The weighted structure of the model was used to 'stabilize' predictions.

Table 6.2.5 BI model equations

		Independent variables (x)							
Function of initial variable (y) with time (t)	**Dependant variables (y')**	**rAmmonia**	weight	**rK+**	weight	**rHb**	weight	**rHkt**	weight
BcAA/AroAA = y't +3.58	rBcAA/AroAA	y' = -0.0016x + 0.0004	0.387	y' = -0.4736x - 0.0521	0.460	y' = 0.2276x - 0.051	0.407	y' = 0.0556x - 0.0489	0.339
Glutamine = y't +152.10	rGlutamine	y' = 0.0832x + 3.6009	0.317	y' = 22.124x + 6.4388	0.400				
Bilirubin = y't +4.53	rBilirubinTOT					y' = -3.8422x + 1.1073	0.564		
Fibrinogen = y't +2.47	rFibrinogen	y' = -0.0004x - 0.077	0.464	y' = -0.1245x - 0.0895	0.536	y' = 0.1973x - 0.0692	0.429	y' = 0.0561x - 0.064	0.657
PT = y't + 10.28	rPT			y' = 1.2325x + 0.7003	0.400				
Antithrombin = y't +91.43	rAntiThrombin					y' = 4.4269x - 1.4852	0.250	y' = 0.9887x - 1.5065	0.267
Factor II = y't +51.65	rFactor II			y' = 2.2772x - 2.0043	0.446				
Factor VII = y't +57.64	rFactor VII			y' = 1.9639x - 2.4117	0.436				
Factor X = y't +74.59	rFactor X							y' = 2.275x - 1.6933	0.479
ALP = y't + 107.3	rALP			y' = 57.521x + 16.998	0.525	y' = -49.387x + 15.048	0.291	y' = -16.927x + 12.141	0.264
AST = y't + 64.4	rAST	y' = 4.4629x + 59.621	0.555						
LD = y't + 330.0	rLD	y' = 6.6529x - 135.8	0.373	y' = 1049.2x + 141.74	0.427				
ALT = y't + 39.7	rALT	y' = 0.1495x + 5.3928	0.282						
Creatinine = y't +86.92	rCreat	y' = 0.1556x - 3.1144	0.355	y' = 49.361x + 1.4321	0.643	y' = -35.25x + 0.1292	0.346	y' = -8.6656x + 0.4563	0.273
Urea = y't + 3.03	rUrea					y' = 0.2484x - 0.0129	0.618		

Notes:

1. The arbitrary inclusion criterion for any particular equation was that there must be a Spearman correlation coefficient of magnitude no less than 0.25 between the dependant (y') and independent variables (x) in question. The empty parts of the table represent those equations that did not meet the inclusion criterion. Some of the outputs are described by only a single input; consequently, these predictions are likely to be less accurate than those predicted by several inputs.
2. The Spearman correlation coefficients were used as weights in determining a summated product for the particular biochemical rate in question.
3. Once a dependant variable's summated value was determined, an absolute value for the biochemical could be calculated from the function of the biochemical raw data with time (first column).
4. The above statistical trends were determined from the data of a total of 12 animals. These were animals that successfully met the criteria of inclusion (table 5.3).
5. The shaded dependant variables (rGlutamine, rCreat and rUrea) were considered for subsequent omission from the model, due to the divergence of predicted values from those measured.

6.2.3.6 Model sensitivity

This was tested using Tornado diagrams and Monte Carlo numerical simulation methods. A selection of the resulting graphs are presented in Appendix D.3.

1. Tornado diagrams [285].
These diagrams display the sensitivity of the output of a model in terms of the numerical range of its input variables. This method assumes Gaussian normality in the input populations. As was expected in this case, the weight appropriated to each of the input variables strongly influenced their effect on the model's output. This was true in both the PI and BI parts of the model and further validated the weighted numerical design of the model.

2. Monte Carlo (MC) simulation [286,287].
Populations of random numbers, either normal on uniformly-distributed, were also used as input to the model. This was done both 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 and graphical projection. In all cases, each of and a combination of the input variables very closely approximated the measured mean values (i.e. < 10 %). However, there was consistently less variation in the predicted values than in the measured population. This may have been due to the limited size of the training population. The effect of using uniform distributions was to slightly under-estimate the output. The model would therefore tend to produce more conservative estimates with non-normally distributed data.

5.2.3.7 Assumptions of normality

The predictive ability of the numerical model is also dependent on the degree to which its input popoultions are normally distributed. To investigate this assumption the measured raw input data was tested for normality using Shapiro-Wilk tests. Despite the small size of the population from which the model's equations had been derived, normality was only

excluded in the derived variables rHb and rAmmo (PI model). Similar to the above, the effect of uniformly distributed populations was to marginally underestimate the measured results. Thus, prediction error would tend to be conservative. In practice therefore, the requirement of clinical interventions would be indicated earlier rather than later (a good thing!).

The above results are presented in Table D.4.1 in Appendix D.

6.2.3.8 Factors affecting BI accuracy

The following four factors potentially determining the prediction accuracy of the BI were identified:

1. The number of independents used to determine each dependent variable (see table D.5.1 in Appendix D).
2. The accuracy of measurement of the independent variable/s. Specifically, the large measurement deviation in the biochemical variables (section 5.2), and
3. The strength of correlation between the variables.

It was consequently expected that the BI would not be as accurate as the PI.

6.2.4 Model Verification

This was performed using a variety of statistical techniques, including analysis of variance (ANOVA), 'relative error' calculation and direct comparison with prospectively acquired, *un-trained* data. For more detail on these techniques refer to Appendix D.6. Briefly,

6.2.4.1 ANOVA

After calculating mean and standard deviations and percentage deviations between all measured and predicted populations it was found that the predicted means very closely approximated the measured means, i.e. < 1%. In certain biochemical variables, such as

Factor X, urea, creatinine and glutamine the percentage error was unacceptably large, indicating their potential exclusion from the BI part of the model.

An ANOVA comparison was drawn between all predicted and measured populations. It was found that the variances were similar although that in the predicted populations tended to be greater. In summary, it was not possible to detect significant differences between the populations in any of the parts of the model. These results were taken as a positive indication of the accuracy of the model.

6.2.4.2 Relative error

For visual and statistical indications of prediction error-range and magnitude the following was done:

The *point error* error (i.e. the deviation of each predicted to corresponding measured value as a fraction of each measured value) was calculated. This was then divided by the standard deviation (std dev) of the measured population to give *relative error (re)*. As *re* approaches zero, the greater the model's prediction accuracy. These results were graphically projected. Significant differences were taken to be where the *point error* exceeded 100 % of the std dev of the measured population. The std dev of the *re* (SD*re*) indicates the predicted *error range*. To overcome potential weaknesses in this method associated with variables demonstrating large measurement variations the measurement range was multiplied with the standard deviation of the relative error (SD*re*) for a quantitative comparison. The larger the returned value, the larger the prediction *error region*. For details of these results please refer to Appendix D.6.2.

In the surgical period (T<0) the PI's point errors all fell within $\pm$ 8 %, and in the post-surgical period (T>0) it fell within $\pm$ 3.5 %. In the BI, only in urea did the relative error fall outside of the 100 % mark. These findings agreed with that previously found. Specifically, the PI was more accurate than the BI. In the latter case this was not

considered critical since its purpose (the BI's) was to have *on-line* estimations for biochemical variables for which there were no sensors.

6.2.4.3 Comparison with prospectively acquired *un-trained* BALSS treatment data

Following the definition of the model, data was subsequently collected during 8 BALSS treatment experiments, including with-cells versus without-cells (control) bioreactor configurations (table 6.2.6). (The evaluation of this data was also previously described in section 5.3)

The PI, based on the rate of change of biochemical variables in the ICU (T>0), was accurate to within less than 10 % in all cases. As expected, the PI was less accurate in the T < 0 period than in the T >0 period and also more accurate in the BALSS with-cells versus without-cells configurations. In the first instance this was likely due to the greater number of absolute-valued, less 'weighted' (i.e. in total, less linear first-order) variables used in the surgical versus ICU periods. In the second instance, the lower accuracy, specifically the survival over-estimation in the cell-free configurations, would seem to indicate that the model was *missing something*, i.e. there were changes in variables that the model was not designed to register. As per our prior evaluation (section 5.3), the likely explanation lay in the observed coagulation problems in those experiments. Indeed, in this model's instance, neither part (T < or > 0) was designed to predict prognosis based on coagulatory variables. This was as a result of the fact that there were no *on-line* sensors for any coagulatory variables at the time. This circumstance has subsequently changed. Since ALF is multi-systemic, a prognosis model should employ (ideally *on-line*) independent variables representing as many of the involved organ-systems as possible.

Importantly, the ischemic animal trials were terminated prior to completion (due to financial deadlines) limiting the amount of data available for the prospective verification of the model. Despite the observed accuracy, a larger data set would obviously have been preferable.

Table 6.2.6 Comparison of predicted to measured *un-trained* BALSS test data with the PI

	Measured survival (hrs)	**Predicted survival**		**Percentage Difference**	
Group		**T<0**	**T>0**	**T<0**	**T>0**
BALSS + cells	20.5	23.7	21.7	+ 15.6	+ 5.9
	29	29.3	28.1	+ 1.0	- 3.1
	30	29.6	32.3	- 1.3	+ 7.7
	24.5	27.3	22.1	+ 11.4	- 9.8
	21	26.9	19.0	+ 28.1	- 9.5
absolute mean	N = 5			11.5	7.2
BALSS – cells	14.5	25.9	25.3	+ 78.6	+ 74.8
	10.6	26.8	18.1	+152.8	+ 70.8
	17.5	26.9	18.7	+ 53.7	+ 6.8
absolute mean	N = 3			95.03	50.8

Note: The sensitivity of the numerical scale should be considered, e.g. one hour is 5 % of 20 hours (the mean survival). The time of death was often measured to only an accuracy of 30 minutes. Thus, predicting to within less than 2 hours in these (rather difficult) experiments qualifies as accurate and validates the prognostic value of the variables used in the model.

6.2.5 Discussion

The prognosis of surviving ALF has steadily globally improved over the last 4 decades. This has been as a result of improvements in the molecular understanding of the disease and intensive care methods [24-26]. As yet, no BAL device has demonstrated sufficient efficacy in the human clinical scenario to lead to a commercially available product. This is despite great efforts in this respect. Artificial liver systems have also not shown improvements in patient survival in randomized clinical trials although improvements in patient biochemistry have often been demonstrated [72,82]. A picture that appears to have emerged is that improving patient biomarkers does not translate to improvements in survival. However, the value of an *on-line* biomarker system is obvious: Knowledge of the degree of progression of a highly acute disease will almost certainly provide a benefit to the clinician and therefore the patient.

In the above regard, a recent study investigating the clinical efficacy of the MARS artificial liver support system employed the MELD score (and the SOFA score, Glasgow coma scale and APACHE II criteria) on ALF patients at admission and at 3 months follow up after a treatment series [269]. It was discovered that the treatments resulted in improvements in the MELD score, but that improvements in survival were not calculable based on the small patient cohort. Importantly, the MELD score is defined in terms of biomarkers and fundamentally assumes a correlation between mortality and the resulting score. By implication, an improvement in biomarkers was associated with an improvement in survival.

A basic review of existing prognostic systems [52-55,239-241,261-269], independent of etiology, reveals that the extensive range of variables that have been employed are mostly of a biochemical nature (table 6.2.7). i.e. they are metabolic biomarkers. Despite extensive historical efforts no single prognostic criteria system has achieved worldwide use (although the MELD score appears to be becoming progressively more universally applied). This circumstance may be due to regional demographic

and etiological differences in ALF, with consequently varying pathogenesis. In general, first world countries have more cases resulting from self harm attempts (e.g. acetaminophen), while third world countries present with more of a viral origin (e.g. HBV). This fact may limit the accuracy and specificity of prognostic criteria employed on different patient groups to those on which they were defined. Investigations into the prognostic value of new/additional clinical variables are ongoing [239-241,267].

Without exception, the *historical* biomarkers of ALF have been identified using multivariate statistical analysis of patient data on admission to the hospital/clinic. An underlying principle seems apparent, namely that several variables representing the multi-systemic nature of ALF should ideally be incorporated into any particular prognostic system. Such an approach would ensure the representation of all physiological systems, even if any one variable failed to demonstrate a routinely high correlation with survival. For example, using variables from the following possible organ-systems may result in a more molecularly representative prognostic model than has been available to date:

1. coagulatory system variables, e.g. INR, PT , clotting factor 5.
2. immunological variables, e.g. IL-6, TNF-α.
3. liver toxin clearance variables, e.g. ammonia, bilirubin.
4. liver synthetic functions, e.g. AFP, complement (C3a, C5a). albumin.
5. kidney function, e.g. creatinine.
6. cerebral functions, e.g. HE, coma, ICP, $SrjO_2$, PI.
7. systemic metabolism, e.g. pH, lactate and/or pyruvate.
8. systemic circulatory and/or pulmonary functions, e.g. FiO_2, serum Na^+, and
9. perhaps a demographic variable, such as age.

Notes: This list is tentative and variables may be added/subtracted following the resolution of their prognostic value. For an explanation of the meaning of abbreviations please refer to table 6.2.1.

Table 6.2.7 Review of variables that have demonstrated prognostic value in ALF

[1]Study/s	Variables																				
	age	pH	AFP	Alb	Factor V	PT	INR	Serum Bili	Creatinine	Urea	Ascites	HE	Serum- Na	HVPG	FiO_2	FOS	Hb	Ammonia	Lactate	Pyruvate	Ph-alanine
KCC [52,53,260]		✔				✔	✔	✔	✔			✔									
[2]Cliché [55,261]	✔		✔		✔							✔						✔			
MELD+modified [262,263]						✔	✔	✔	✔				✔	✔							
CTP+modified [264,265]				✔		✔		✔			✔	✔									
RFH [266]								✔		✔					✔	✔			✔		
[3]Dabos *et al* [267]																	✔		(✔)	✔	✔
Bernal *et al* [268]																			✔		
[4]Bhatia *et al* [239]	(✔)	(✔)		(✔)		(✔)		(✔)				(✔)						✔			
Clemmesen *et al* [240]																		✔			
[5]Bernal *et al* [241]	(✔)							(✔)					(✔)					✔	(✔)		

Footnotes:

1. The above studies were selected to represent prognostic criteria for all causes of ALF (i.e. mostly paracetamol, viral, cirrhotic). The table is not intended as an exhaustive review, and it does not provide the parameter values used in each system. Sequential organ failure assessment (SOFA) and acute physiology and chronic health evaluation (APACHE) III scoring systems are not included since they were designed with *all*, rather than only liver forms of organ failure in mind.
2. Additional independent predictors of poor prognosis: presence of coma, hepatitis B virus marker (HBsAg)
3. Additional independent predictors of poor prognosis: alanine, acetate, calcium, lactate.
4. Additional independent predictors of poor prognosis: pH, age, HE, cerebral oedema, bilirubin, albumin, PT, bicarbonate.
5. Additional independent predictors of poor prognosis: high MELD score, CVVHF, vasopressors, bicarbonate.

Entries in brackets indicate variables that had prognostic value but were not primarily studied.

6. Abbreviations: KCC = King's College criteria (London), Cliché criteria (France), MELD = Model for end-stage liver disease (US), CTP = Child-Turcotte-Pugh criteria, RFH = Royal Free Hospital criteria, HVPG = hepatic venous pressure gradient, INR = international normalized ratio, AFP = serum α-fetoprotein, ser- = serum, Ph-ala = phenyl alanine, FiO_2 = % inspiratory O_2 concentration, FOS = Failing organ system

One variable of particular interest is arterial ammonia owing to its historic absence in prognostic criteria. It is surprising that despite impressive progress in understanding the central role of ammonia in the pathogenesis of ALF [35-40], studies have only recently undertaken to investigate its importance as a biomarker with prognostic value in ALF [239,241]. Interestingly, Bernal *et al* (2007) [241] found that their uni- and multi-variate models were more accurately predictive of survival when combined with the MELD score, which is itself a multi-systemic prognostic system. In any case, the data of the animal model on which the second of the above animal studies was based (section 5.2) clearly demonstrated a strong correlation between rising arterial ammonia levels and the duration of survival. However, this may be unsurprising in view of the toxicity of the ischemic model.

On-line ICU patient monitoring technologies and time-series data analysis methods have been available for some time [291-295], however, only in recent years has there been any effort to make prognosis scoring systems 'dynamic'. For example, a recent study demonstrated that the prognostic accuracy of sequential organ failure scores (SOFA) of patients in an ICU was significantly improved if the trend or change in the daily evaluations were incorporated into subsequent mortality prediction models, rather than using only the single admission score as has been done to date [296,297]. There seems little doubt that a mortality model's value, both to patient and clinician, will increase with the regularity with which it is updated.

Both the UML and numerical methods employed in this study aimed to model the multi-systemic character of ALF. The UML has repeatedly been indicated as well-suited to data integration in systems biology. To paraphrase Rouquié *et al* [276-278], firstly, 'an entity considered to be a system can be represented as the interface between an internal and external environment on which it is acting and in which it is evolving', and secondly, 'its behaviour is describable as a state trajectory in a time, space and form frame'. The UML is useful as a metamodel in that it can integrate the data of underlying models that may be of such complexity that it is impossible to contain them all in a single model. Take a genomic, proteomic, cellular, organ and metabolic system as a single hierarchical example.

On a minimal level, in this study the UML was useful for specifying a hypothetical ALF-patient-system in terms of the variables (data) composing its organ-system structure, and perhaps more importantly, its functions in terms of a state trajectory in time. Obviously, modeling a complex system such as ALF is error-prone. At this stage a relatively greater value of the proposed model/s lies in their methodological foundation/s rather than the particular clinical instance. In the future it will be desirable to include additional animal and human data.

The numerical approach above resulted in a summed, weighted multivariate combination of absolute-valued data representing the surgical intervention and the rates of change of systemic circulation and biochemical data for which *on-line* detection equipment existed. The variables that were selected had previously established prognostic value in ALF, linearity over time and the availability of *on-line* sensing equipment. The benefits of this approach were:

1. Non-linear analysis methods, such as Kohonen self-organizing maps (SOMs), in the neural network toolbox in Matlab (www.mathworks.com), did not produce useful results on the small data sets.
2. Verification demonstrated that first order (linear appreciation/depreciation with time) assumptions were justified in the employed variables. This meant that based on the duration of the experiments (> 20 hours), the more time-linear and accurate latter part of the numerical model would be more extensively employed than the former.
3. The model satisfied Occam's razor in that a conceptually simple method modeled a process for which no *a priori* descriptive equations existed. The relative ease with which new variables may be incorporated into the numerical model is an attractive part of its design. For example, in future modeling iterations it may be attractive to investigate more biochemically oriented variable sets and to include transcranial doppler (CBF, PI, $SrjO_2$, CMR, CPP), cerebral microdialysis or intracranial pressure (ICP) variables (which are good predictors of HE in ALF [26, 297-299]).

4. The linearization about some point of interest along a non-linear function is a common procedure in bioprocess technology (although the regularity of *on-line* measurements then determines the model's accuracy [11,279-283]).

It was noteworthy that numeric verification using an untrained dataset demonstrated accuracy in the PI model's ability to predict the duration of survival, specifically in the BALSS with-cells group and particularly in view of the numerical method's multi-systemic representation of ALF. However, larger sample sizes would obviously have preferable.

Regarding the biochemical indicator however, although intrinsically possible and even relatively accurate on its training dataset, the BI is understood to be somewhat contrived. Prior studies have found little correlation between independently measured metabolic biomarkers in ALF. For example, only ammonia and bilirubin levels were found to correlate ($p = 0.01$) in a study investigating the prognostic value of arterial ammonia [239]. This is why not all the BI's results were provided and why additional verification was not carried out on the un-trained treatment dataset. It should be recalled that the BI is a 'software sensor' [279,283] that was defined to provide indications of biomarkers in the absence of *on-line* equipment. It is obvious that an *on-line* approach remains the ideal solution.

In terms of system implementation, the proposed numerical model is well suited to application as a 'Kalman filter' (KF). This is a linear state estimator used for calculating the value of bioprocess variables that are normally not measurable *on-line* (e.g. the biomass or product/substrate concentrations in a production bioprocess [11,279-283]). This method involves continuously updating a pre-defined process model with *on-line* sensor measurements for independent variables. An effective KF has two basic requirements:

1. The process model must be accurate and insensitive to measurement errors, and
2. The system and measurement noise should be determinable.

This raises the following points:

The proposed model is unavoidably reliant on the accuracy of sensors for the independent variables. In fact, the variables were chosen owing to the commercial availability of *on-line* sensors. Ensuring measurement accuracy is possible using noise-filtering techniques, such as first or second order filters or a moving average of measurements. A data integration/processing system with a graphical user interface (GUI) would consequently also be necessary. In this respect, an active virtual state estimator, as represented by figure 6.2.2, would be a useful component of a GUI.

Bioprocesses are harsh environments for sensors in that protein fouling of surfaces may cause measurement problems. A solution to this is sample extraction from the process loop with a subsequent buffer-washout phase, as is already used in flow injection systems [270,271]. For example, clinical microdialysis (table 6.2.8) is a technique designed to monitor tissue chemistry (often cerebral) during or after pharmacological, physiological and surgical interventions. Very small diameter sterile catheters are employed; these have semi-permeable membranes to allow the appropriate tissue fluid to pass through. Sample volumes are also kept very small to avoid patient haemodynamic instability.

Table 6.2.8 Examples of commercially available FI systems which are potentially attractive in ALF

Device/s	Primary application	Chemistries	Sample volume	Cost	Reference
YSI7100 MBS	Bioprocess monitoring and control	glucose, lactate, glutamate, glutamine, ammonium, and potassium.	10-15 µl	±R 300 000 incl. initial buffers and calibration fluids	www.YSI.com
CMA600 analyzer	Neuro-microdialysis	glucose, lactate, pyruvate, glutamate, glycerol, and urea.	0.5 µl	±R 500 000 incl. initial buffers and calibration fluids	www.microdialysis.se

Since there are currently no bioprocess monitoring systems designed for ALF, the state estimator in this study was designed for use with equipment normally available in surgical and ICU settings: For example, electrocardiographic (ECG) instruments and arterial blood gas (ABG) technology. In the post-surgical part all but ammonia may be detected using an ABG machine. A variety of sensors for ammonia are commercially available (www.spectronic.co.uk). A sensor for creatinine is not yet commercially available, however, research has been underway to produce one [304]. Perhaps, the most ideal sensors are *biosensors* employing enzymatic amperometric or optical methodologies for detection [305-308].

Assuming the success of modeling ALF in animals, doing so in humans may present more of a challenge. Standardized animal models are 'pure' images of ALF and the interventions are mostly minimized. Interventions in the human scenario tend to be excessive in the hope of saving the patient's life. Linear approaches are therefore likely to be less successful due to these complicating effects. However, extensive clinical 'know-how' exists and this is obviously the basis for successful support of ALF patients. 'Knowledge-based' combined with structured non-linear models may therefore be of value in the human scenario. The UML, as an object oriented meta-modeling method, is likely to continue being useful in integrating these [273,309,310].

6.3 Thoughts and recommendations

In the above two studies, a compartmental pharmacokinetic and an *on-line* prognostic model were investigated. These provide information regarding BAL system performance during treatments, and *on-line* biomarkers and prognosis respectively.

In terms of the former, although the pharmacokinetic modeling approach is a necessary and desirable part of developing a BAL, it provides predictions regarding the clearance or production of only single substances at a time and only after rates of production and or clearance have been measured in *in vitro* and *in vivo* experiments. As has become apparent, the clinical syndrome of ALF is a complex, multisystemic process, which cannot be limited to only individual clearance and/or production variables. The addition of bio-analytical monitoring tools designed to investigate changing clinical variables that participate in known or suspected ways in ALF may help to prevent assumptions regarding pathogenesis and the efficacy of any potential treatment.

Subsequently, a bioprocess state estimator was proposed for a porcine surgical ischemic model of acute liver failure. Its purpose was to create an *on-line* system providing indications of experimental animal prognosis and biochemistry. In the instance described above the model's ease of extension and numerical accuracy would suggest that the diagrammatic and statistical modeling methods were indeed successful. That is, the selected independent variables did accurately determine the particular dependent variables. This also implies that clinical interventions should aim to minimize or reverse changes in these variables to demonstrate the benefit of the treatment. For example, blood sampling should be minimized to prevent haemodynamic instability and the accumulation of ammonia should be minimized using an appropriate means of clearance (e.g. an artificial toxin clearance column and biochemical ammonia minimization strategies). Unlike the pharmacokinetic model, the value of this model resides more in clinical patient management than in BAL design.

The following additional thoughts are relevant:

6.3.1 Refining prognostic models

In view of the fact that studies investigating new variables and those comparing existing systems have continued, one can assume that the final word on prognostic criteria for ALF has not yet been written.

The complexity of ALF will doubtless require several collaborative modeling attempts prior to acceptance as 'complete'. It is therefore sensible, in principle, to proceed with ALF process modeling in animals before attempting application in the human context. Since the outcomes of previous UP-CSIR BALSS trials were apparently affected by the toxicity of the ischemic model (section 5.3) future trials aim to use the anhepatic model [5,220,226,230] to hopefully 'unmask' the benefit/s of the treatment as previously mentioned. As stated, the anhepatic is a type of 'control' for the ischemic, i.e. subtracting the rates of endogenous toxin accumulation will indicate the contribution/extent of hepatic necrosis. This information is useful in the numerical definition of an ALF process model. Additionally, it would be desirable to include several new coagulatory, immunological and cerebral variables (table 6.2.1), in future experiments.

In the human scenario thereafter, spontaneous liver regeneration and clinical interventions aimed at improving prognosis will likely alter biological processes that appeared to linearly appreciate/depreciate with time in the animal scenario/s. Thus, future modeling efforts may benefit from larger sample sizes and non-linear techniques. Pattern recognition and rule-based artificial intelligence systems have successfully been applied to model complex medical problems [291-293]. For this reason, it is probable that a human process model of ALF would benefit from a hybrid *learning and rule-based* design approach [294] which has (apparently) not been attempted before.

6.3.2 Non-linear and multivariate regression experiments

After defining the ALF process model on the 'ischemic-standardization' data, the prospective acquisition of the BALSS treatment data created the possibility of re-examining the new and larger raw dataset with additional analysis and modeling efforts:

The aim was to explore the possibility of there being a prognostic model contained in the data that was composed of variables that were more biochemically-oriented. In particular, variables more similar to those identified in the review of prognostic studies (table 6.2.7) than those defined in the above numerical modeling instance. This effort was in part focused on finding prognostic variables that were preferably not measured by either ABG or ECG instruments since these would in any case be in an ICU.

Kohonen self organizing maps (SOMs in Matlab) were initially re-employed and were once again found to be ineffective, likely due to the still too small sample size. Incidentally, SOMs are a single-layer form of artificial neural networks (ANN [11,311,312-316]) which are useful for indicating multidimensional relationships in an intra-related data space. The problem in this case lay in the biochemical sampling interval: The laboratory variables were sampled 4-hourly to minimize costs and to prevent animal haemodynamic instability (as a result of blood loss). Although the ABG and ECG data was collected on a much higher frequency, including this into the final 'summated dataset' required limiting the sampling interval to the lowest common denominator (i.e. only the data points of all variables on the 4-hourly intervals). SOMs were simply not the appropriate tool for the task.

Subsequently, an un-weighted least squares linear multivariate regression (as is available in SPSS 17.0) was performed on the raw data. Combined as a single set, the following variables were selected: pH, bicarbonate (HCO_3), potassium (K^+), hemoglobin (Hb), lactate, pyruvate, ammonia, bilirubin, creatinine, urea, prothrombin time (PT) and the clotting factors 2,7 and 10. Thereafter, a process of stepwise exclusion of variables not demonstrating significant p-values in the combined-model form was followed to determine the final multivariate model. On completion the following remained, K^+, ammonia, bilirubin, creatinine, PT and urea. The individual p-values were all below 0.05, in fact, the highest was 0.011 for bilirubin. The similarity of this model to the variables identified in the review (table 6.2.7) is indeed noteworthy, particularly to the studies of Dabos *et al* [267], Bhatia *et al* [239] and Bernal *et al* [241].

The difference between this (multi-variate) model and the previous numerical model (state estimator) lies in their derivation methods. The variables in the state estimator were mean rates of change (the gradients) calculated from the first-order relationships of each of the variables of interest in each of the animals, i.e. they related the duration of survival to 'derived' data. The multi-variate model, on the other hand, directly related the raw data to time. The value of this multivariate model lies in demonstrating that the assumption previously made was justified, namely that the rates of change (first derivatives) of the above listed variables are indeed linearly related to the duration of survival. This validates building a dynamic *on-line* prognostic system based on the rates of change of appropriately selected variables. As stated before, such a system will formally require either *on-line* sensors for all of the variables or a process model [300-303] with data inclusion methods for the *off-line* (laboratory) variables.

6.3.3 A UML meta-model to combine the state estimator and the BALSS pharmacokinetic model

A variety of systems have been used to model biological systems, the choice of which is normally determined by the postulated underlying structure of the system, for example, petri nets, boolean switching nets, electronic circuits or ANNs. Each of these systems have advantages and disadvantages determined by the quality and quantity of data, the understood complexity of the system and the degree to which this requires accurate emulation [302,303,315-317].

With this in mind, it is interesting to compare the above state estimator with the pharmacokinetic compartmental model (table 6.3.1). What becomes apparent from the comparison is that the two systems complement each other in that they are designed to examine system pharmacokinetic performance and the patient's prognosis (state trajectory) respectively.

Table 6.3.1 Differences between the state estimator and the compartmental model

Aspect	State estimator	Pharmacokinetic model
Purpose	to create an *on-line* monitoring system that predicts patient prognosis and biochemistry.	*off-line* examination of the impact of system design factors on the efficacy of metabolite production/clearance.
Value	treatment efficacy and financial savings	determining BALSS performance and system design
Assumptions	linearity with time of change in independent variables. No compartmental mass conservation assumption.	conservation of mass of particular metabolite/s in well-stirred compartments.
Types of equations	rates of change of linear functions	ordinary differential equations (ODEs)
Derivation method	empirically derived relationships between *in vivo* survival and biochemistry, and *on-line* biochemistry with *off-line* biochemistry.	*in vitro* measured rate of clearance of metabolite (e.g. ammonia) by bioreactor and *in vivo* measured production by patient
Types of variables	any systemic variables or blood metabolites/biochemicals that may be measured *on-line*.	*off-line* blood metabolites requiring laboratory determinations.
Number of variables	several variables simultaneously.	one variable at a time.
Sensitivity to measurement error	insensitive	sensitive

Combining these two systems might involve their interaction in terms of in and outputs (i.e. output for one may be input for the other). As previously stated, the UML is attractive for combining models of different levels into a meta-model without internal conflict. In this regard, there are convenient extensible markup language (XML) procedures to convert UML diagrams to code.

To achieve this proposed systems integration the following meta-model may be proposed:

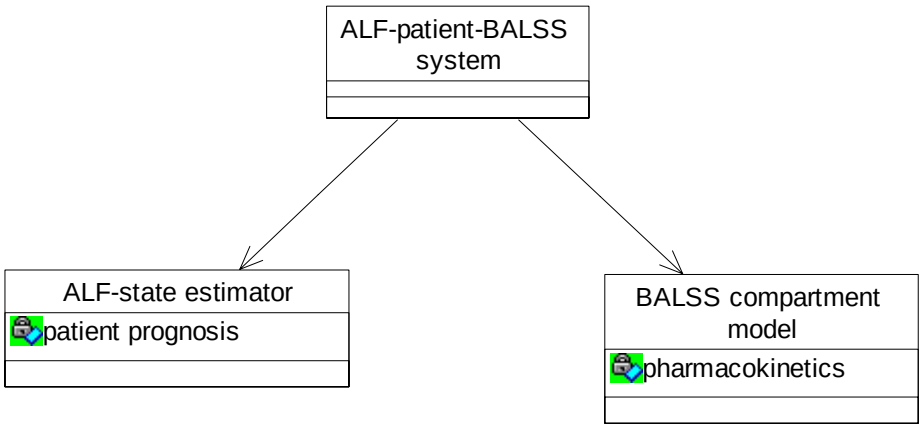

Figure 6.3.1 UML metamodel of the combined ALF-patient-BALSS compartment models. The inheritance of the meta-model includes all the attributes and functionality of the two underlying models.

Obviously certain basic requirements would need to be met in terms of the implementation of such a system. For example, in addition to reaching a more final version of the ALF-patient state estimator, a state trajectory for the compartment model, i.e. the pharmacokinetic dynamics of the BALSS, would also require definition. The result would be a combined ALF-patient-BALSS state model with behaviour inherited from the attributes of the two underlying systems.

In this regard, suitable in and outputs linking the two models would need to be selected. Although the hardware of a bioprocess monitoring system is an independent system (which need not communicate with any particular model), any variables selected to enable communication between the above models would need to match the criteria for inclusion in both i.e.:

1. drugs or metabolites that are alternately cleared and/or produced by the bioreactor and patient respectively,
2. The existence of sensors and equipment for their o*n-line* measurement during experiments
3. As high a correlation, or ‘weight’, with the dependent variable as possible (i.e. a clinical association of relevance in the animal ALF model).
4. As linearly appreciating/depreciating over time (i.e first-order) as possible.

For example, assuming the *on-line* detection of ammonia, potentially in various sites in the system (e.g. arterial in the patient and in the various compartments of the BALSS), the measured data could function as input for both pharmacokinetic and prognosis models. Depending on the use of an animal or human treatment model other variables may also match the above characteristics. In an anhepatic model a variety of plasma proteins (e.g. coagulation factors) and endotoxins may be suitable candidates.

As mentioned previously, it is important to realize that the above models are iterative instances and will benefit from extension in the future. For example, the pharmacokinetic model may additionally include; an artificial toxin clearance device, with known toxin clearance dynamics over time, or the effect of supplementing the patient with blood plasma or albumin. The prognosis model could also be extended to include several of the variables (listed in table 6.2.1) not previously measured. In either case, an appropriate *on-line* monitoring system that is independent of the underlying models will remain a formal requirement.

Finally, assuming both models are well understood, an *in silico* meta-model of the above could be used to simulate the likely behaviour of the combined system. A virtual system such as this would be highly attractive in terms of BALSS system design finalization and therefore time and expense savings prior to proceeding to human clinical trials.

6.3.4 Additional notes on bioprocess monitoring systems

Of the previously mentioned FI systems the CMA system is more attractive than the YSI system for the following reasons:

1. Several input channels are available in the CMA system. Thus, the cerebral biochemical data of several patients along with data from any additional devices may simultaneously be monitored.
2. In clinical, as opposed to laboratory bioprocess systems, feedback control is not required. The CMA device has a data integration software system (ICU Pilot) that is able to simultaneously display trends in several systemic variables (ECG, EEG, ICP, MAP etc) and the measured biochemicals.

3. Since the sampling catheters may be adapted for both cerebral interstitial-fluid and blood plasma, in a BALSS treatment, one catheter could be used to monitor brain chemistry while another monitors plasma chemistry. In this case the sterility issues regarding the *in situ* application of a clinical device have been adequately attended to. This is not so with the YSI machine although a neurological application has not been excluded. In addition, in the ischemic experiments HE, which is a diagnostic characteristic of ALF, could not be diagnosed on the basis of EEG results as these are known to be influenced by sedation, nor by ICP since it was not monitored. Instead, HE was diagnosed via alterations in Fischer's ratio, which relies on after-the-fact biochemical analyses of plasma amino acid ratios. The CMA machine will allow an *on-line* diagnosis of HE via cerebral biochemical indicators potentially integrated with an ICP measurement system. The concentration of the cerebral amino acids may also be compared to the plasma amino acids, as measured in the BALSS-patient circulation loop, and this is likely to provide valuable prognostic information.

Assuming the development of a data inclusion system (Kalman filter) a variety of *off-line*, biochemistry analyzers are ave recently become commercially available that would provide prognostically valuable data for the ALF process model. For example, the Diagostica Stago STA-compact device (www.stago.fr) that measures a variety of coagulatory variables (PT, APTT, fibrinogen, thrombin time, clotting factors and antithrombin) or the large range of Siemens analyzers that perform immuno-assay, chemistry and hematology analyses (www.siemens.com/diagnostics). In addition to the flow injection technology mentioned up to this point, there is also a possibility of incorporating new high-throughput molecular technologies with electronic signal transduction, potentially enabling measurement updates several times a second [2]. The latter will obviously require specification of the correct sensors.

In the course of developing the UP-CSIR BALSS a FI sub-system was designed to take samples from the re-circulating plasma circuit during the course of a treatment (figure 6.3.2). The sampling volume is small, 2 ml, and the sampling interval is programmable: Once every 30 minutes would be sufficient for statistical purposes in an animal model and would also have a minimal haemodynamic impact. Each sampling cycle is followed

by saline washout from an off-line reservoir. However, due to inconvenient electrode sterilization and calibration procedures, the system has not yet been clinically applied. A stand-alone system such as the CMA device, in which all of the problems associated with a clinical application have been solved, remains a more attractive solution.

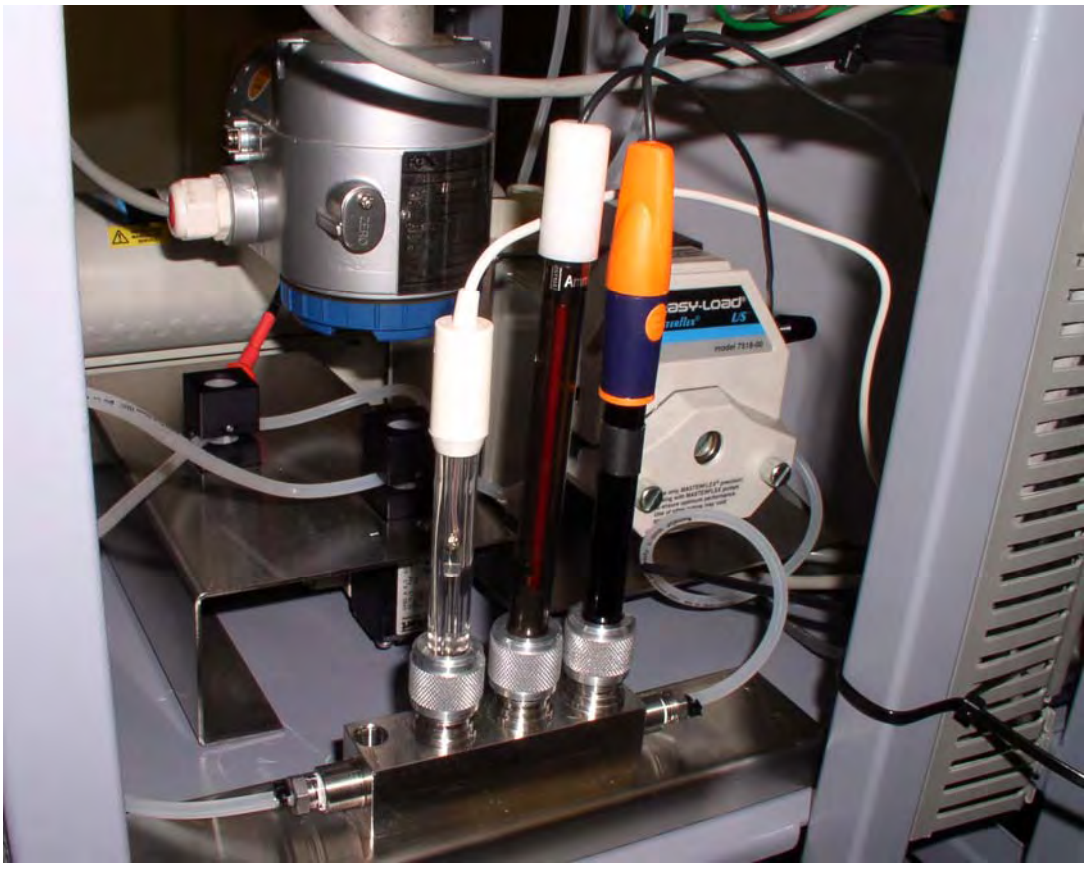

Figure 6.3.2 UP-CSIR BALSS FI sampling manifold with ion-specific electrodes. From left to right carbonate (HCO_3^-), potassium (K^+) and ammonium (NH_4^+) electrodes, with peristaltic pump and controller in the rear.

The considerations in developing a bioprocess monitoring device for a BALSS are likely to be the same as those for renal dialysis technology: The treatments are (and will be) very expensive and one possible way to meet these costs is to improve the diagnostic and treatment competence of the system. A system that integrates the signals of several monitored variables will improve the understanding of the links between haemodynamics, metabolism and physiological regulatory systems and will therefore improve diagnosis and treatment regimens [316].

A monitoring system such as the proposed state estimator could potentially function as a monitoring device in any similar bioprocess. For example, in laboratory toxicological, biotransformation and drug screening research, primary hepatocytes and non-parenchymal co-cultures are a popular *in vitro* model owing to the resemblance of the system to an *in vivo* circumstance [316]. The cost of process development accounts for a large fraction of bringing a drug to market. Thus, there is also a strong economic incentive for improved monitoring tools in the bioprocess engineering market [317]. The critical problem remains a paucity of suitable, affordable sensors and integrated monitoring tools.

7. SUMMARY AND CONCLUSION

"It would be an unsound fancy and self-contradictory to expect that things which have never yet been done can be done except by means which have never yet been tried".

Francis Bacon, Novum Organum VI, *1620*

In evaluating the overall success of this thesis, it is instructive to examine the recommendations for future research made in a recent US-based, acute liver failure workshop, Lee *et al* (2008) [243], paraphrased as follows:

- Continued prospective monitoring to identify epidemiological trends in ALF.
- Use of new molecular methodologies to study ALF (especially 'indeterminate' cause ALF), including, genomics, proteomics, metabolomics etc.
- More basic research into ALF and multiorgan failure pathogenesis.
- Initiation of prospective, well-designed clinical trials for various ALF therapies.
- Improvement in predictive markers, modeling of prognosis and identification of new biomarkers. (This task was also reiterated in a subsequent independent study, Freeman *et al* (2009) [318]).
- More basic research in hepatocyte and stem cell growth and proliferation for the purpose of a therapeutic cell source.
- Continuance of *in vitro* and animal studies for BAL devices, to enable eventual human trials.
- Additional tissue engineering work to develop an effective BAL device for humans.

Importantly, the purpose of this thesis was to present and evaluate a variety of types of models and in this respect the following was done and/or achieved:

- After reviewing the clinical and biological background of acute liver failure and bioartificial liver technology and describing the development of the UP-CSIR BAL,
- Three studies involving *in vitro* lab-scale models of the BAL were presented and evaluated: The first established a sterile, numerically large-scale cell source for

seeding BAL bioreactors. This model remains highly successful, despite its regionally limited application due to the xenogenic primary cell origin. The second study successfully demonstrated bioreactor metabolic function, but also revealed methodological difficulties associated with the use of the perflurocarbon O_2 carrier. In response, the third study employed novel methods such as radio-transparent bioreactor materials and positron emission tomography. In so doing, it demonstrated that the O_2 carrier did indeed benefit bioreactors under hypoxic (ALF-like) treatment conditions. The cost and metabolic appropriateness of transformed cell sources were identified as factors at least partially responsible for preventing BAL-device entry into the commercial arena. New research directions, including genetically engineered transgenic and chimeric animals, were mentioned as promising possibilities.

- Two studies involving *in vivo* animal ALF models were presented: The first successfully demonstrated that IV injected PFC was not toxic in either healthy or liver-injury scenarios, and thus, a potential PFC leakage would not be toxic or hinder the recovery of a severely *liver-compromised* patient undergoing a BAL treatment. The second clinically standardized a large animal surgically-induced ischemic model of ALF in which it was possible to statistically identify prognostically important clinical variables (such as ammonia). However, the unstable and multi-systemic nature of the model was also recognized and this contributed to the inconclusive demonstration of efficacy in the clinical evaluation of the UP-CSIR BALSS subsequently described. Despite this, valuable information resulted, including: parameters for BAL system re-design (such as including an artificial toxin device), the benefit of using alternate animal ALF models and the measurement of additional/new prognostically important clinical variables.
- Two mathematical modeling studies were presented: The first, a mass-balance pharmacokinetic compartmental model using actual *in vitro* and *in vivo* data measured in the studies above, simulated the BAL-patient system in a clinical treatment. Valuable BAL design and operation information resulted, including the minimal requisite cell quantities, exchange and circulation rates and (once again) incorporating an artificial toxin clearance device. The second model was an ALF-

bioprocess state estimator defined using conceptual modeling methods (UML) and a weighted-multivariate numerical approach. This model proved accurate on both 'trained' and prospectively collected 'un-trained' in vivo data in the large animal and BAL clinical experiments described above. However, the benefit of larger sample sizes in future studies was identified. Nonetheless, the model proved in principle that it is possible to create an *on-line* clinical monitoring device with ALF prognostic prediction or 'state estimator' capabilities. The means of implementing such a device, additional non-linear and multivariate statistical experiments conducted on the animal data, along with shortcomings and potential improvements to existing prognostic models were discussed. Based on the complimentarity of the above two models, a method for potentially combining them into a single *in silico* model with the benefits of both was discussed.

On at least a scientific level, it is safe to state that the models defined in this thesis achieved their purpose, specifically to generate information regarding BAL system design and clinical and metabolic performance and thereby to facilitate development in the technology. At least half (particularly the latter half) of the list of Lee *et al* (and Freeman *et al*) above was addressed.

Globally, on the other hand, owing to the many variations in BAL design, the cost and technical challenges associated with the requisite studies, and (perhaps as a result) the fact that the literature is littered with examples of initial enthusiasm followed by disappointing outcomes; It might also be said that additional work is required in all of the segregated sections (*in vitro, in vivo*, and mathematical models), before the technology will proceed beyond a pre-commercial developmental stage. Since this is clearly not a *fait accompli* today, in this thesis thoughts and recommendations regarding research progress were provided per section rather than all being lumped into this conclusion. I hope this has been helpful rather than a hindrance to the reader.

Finally, it seems illogical when considering the extensive previous efforts, that the obstacles remaining in the path to BAL commercialization will be solved using repetitions of historical strategies. In agreement with other authors [1,2,9,17,243], it is

probable that developing a clinically effective device will require ongoing innovations and the integration of the new with existing technologies. In particular, aside from the stated requirement of a cell source that meets the needs of all regulatory-authorities, it is probable that mathematical modeling, (in for example, bioreactor design optimization, BAL system performance, biomarker identification and *on-line* clinical and prognosis monitoring amongst others) will enjoy greater application than has occurred in the past. The expense of clinical trials certainly validates *in silico* modeling.