# Prediction of the Performance of Human Liver Cell Bioreactors by Donor Organ Data

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**Abstract.** Human liver cell bioreactors are used in extracorporeal liver support therapy. To optimize bioreactor operation with respect to clinical application an early prediction of the long-term bioreactor culture performance is of interest. Data from 70 liver cell bioreactor runs labeled by low (n=18), medium (n=34) and high (n=18) performance were analyzed by statistical and machine learning methods. 25 variables characterizing donor organ properties, organ preservation, cell isolation and cell inoculation prior to bioreactor operation were analyzed with respect to their importance to bioreactor performance prediction. Results obtained were compared and assessed with respect to their robustness. The inoculated volume of liver cells was found to be the most relevant variable allowing the prediction of low versus medium/high bioreactor performance with an accuracy of 84 %.

## 1 Introduction

Liver cell bioreactors are being developed and used for temporary extracorporeal liver support [1, 2]. Primary human liver cells isolated from discarded human organs are inoculated and cultured in these bioreactors. The 3D liver cell bioreactor investigated here consists of a system of interwoven capillaries within a special housing that serve medium supply and removal as well as oxygenation of the cells that are cultivated in the inter-capillary space of the bioreactor. This bioreactor mimics conditions close to those in the liver organ *in vivo*. It was shown that primary human liver cells obtained from discarded human livers that were explanted but not suitable for transplantation reconstitute to liver tissue-like structures after inoculation into the bioreactor [3, 4].

The design of bioreactor operating conditions that support the long-term maintenance of liver cell functionality is of great importance with respect to the bioreactor‘s clinical application. In previous work, data mining and pattern recognition methods were applied to extract knowledge from bioreactor operation data in order to enable

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the prediction of the long-term performance of the human liver cells in the bioreactor based on early culture data [5]. Using fuzzy clustering and rule extraction methods, the kinetics of galactose and urea over the first 3 culture days were found to be the best single predictors. In addition, kinetic patterns of the amino acid metabolism over the first 6 culture days and their relation to the long-term bioreactor performance were identified and described by different network models (correlation networks, Bayesian networks, differential equation systems) [6]. However, these results alone do not allow to draw conclusions with respect to the causes of the observed differences in the metabolic performance of the bioreactor cultures. These differences may in particular be due to donor organ properties and/or differences in organ preservation, cell isolation and cell inoculation prior to bioreactor operation. This paper presents results obtained by statistical tests and machine learning methods to quantify relations between donor organ and cell preparation characteristics and bioreactor performance.

## 2 Material and Methods

### 2.1 Cell Isolation and Bioreactor Culture

Cells for bioreactor inoculation were isolated from 70 human donor organs that were excluded from transplantation due to organ damage (steatosis, fibrosis, cirrhosis or other reasons). The organs were preserved at 4°C for varying time periods for the transport from the donor clinic to the Charité Virchow Clinic. Cell isolation from these organs was performed with the approval of the Deutsche Stiftung Organtransplantation (DSO) and the local ethics committee using a five-step enzyme perfusion technique as described elsewhere [4].

Cells were inoculated into the bioreactors immediately after isolation and cultured in the systems under standardized perfusion and oxygenation conditions. The culture period was one day to 60 days. The bioreactor culture performance was assessed on the basis of biochemical variables that were measured daily in the culture perfusate (see 2.2).

### 2.2 Data

A data set $x_{i,j}$ ($i = 1,\ldots, I$; $j = 1,\ldots, J$) for 21 metric and 4 categorical variables ($I = 25$) characterizing donor and organ properties as well as organ preservation, cell isolation and cell inoculation of $J = 70$ bioreactor runs was analyzed (Tables 1 and 2). For some metric variables $i$, a number of values was missing (70-$N_i$, Table 1).

Each run was labeled by $L_j \in \{\text{L, M, H}\}$ denoting 'low', 'medium' and 'high' performance, respectively, categorizing the long-term maintenance of the functionality of the liver cells in the bioreactor culture. 18, 34 and 18 runs were labeled L, M and H, respectively. This performance had been assessed by an expert based on the biochemical variables that were measured during the bioreactor operation quantifying enzyme liberation, glucose and lactate metabolism, galactose and sorbitol uptake, ammonia elimination, urea and albumin production and amino acid metabolism.

**Table 1.** Metric variables characterizing donors and organs, organ preservation, cell isolation and cell inoculation (BMI – body mass index, GGT – gamma glutamyltranspeptidase, LDH – lactate dehydrogenase, ALT – alanine aminotransferase, AST – aspartate aminotransferase, GLDH – glutamate dehydrogenase, AP – alkaline phosphatase, PS – preservation solution; Min – minimum value, Max – maximum value, $N_i$ – number of available values)

| Variable | Unit | Min | Max | $N_i$ |
|---|---|---|---|---|
| BMI (of the donor) | $kg \cdot m^{-2}$ | 21 | 39 | 68 |
| Weight (of the donor) | kg | 55 | 140 | 70 |
| Height (of the donor) | cm | 155 | 195 | 68 |
| Age (of the donor) | a | 20 | 79 | 69 |
| GGT (in the donor plasma) | $U \cdot L^{-1}$ | 6 | 1075 | 66 |
| LDH (in the donor plasma) | $U \cdot L^{-1}$ | 71 | 2013 | 47 |
| ALT (in the donor plasma) | $U \cdot L^{-1}$ | 5 | 647 | 70 |
| AST (in the donor plasma) | $U \cdot L^{-1}$ | 3 | 405 | 70 |
| DeRitis (quotient AST/ALT) | - | 0.21 | 5.83 | 65 |
| Bilirubin (total bilirubin in the donor plasma) | $\mu mol \cdot L^{-1}$ | 0.38 | 133 | 67 |
| Urea (in the donor plasma) | $mmol \cdot L^{-1}$ | 1.40 | 91 | 64 |
| Preservation_Time (of the organ) | h | 2.50 | 27 | 69 |
| Organ_Weight | g | 997 | 3378 | 70 |
| LDH_PS (LDH in the preservation solution) | $U \cdot L^{-1}$ | 11 | 5310 | 57 |
| AST_PS (AST in the preservation solution) | $U \cdot L^{-1}$ | 3 | 2110 | 57 |
| GLDH_PS (GLDH in the preservation solution) | $U \cdot L^{-1}$ | 0 | 29 | 57 |
| AP_PS (AP in the preservation solution) | $U \cdot L^{-1}$ | 0 | 19 | 56 |
| Remaining_Mass (of the organ after cell isolation) | g | 179 | 1344 | 66 |
| Dissolved_Mass (of the cells) | % | 20 | 89 | 67 |
| Viability (of the cells) | % | 30 | 85 | 67 |
| Inoculated_Volume (of the cells) | mL | 144 | 800 | 67 |

**Table 2.** Categorical variables characterizing donors, organ preservation, cell isolation and bioreactor culture performance (f – female, m – male, UW – University of Wisconsin solution, HTK – histidine-tryptophane-ketoglutarate solution, Coll. – collagenase P, HSA – human serum albumin)

| Variable | Categories | Distribution |
|---|---|---|
| Gender (of the donor) | {f, m} | [33, 37] |
| Preservation_Solution | {UW, HTK, Celsior, -} | [36, 25, 3, 6] |
| Digestion_Enzyme | {Coll., Liberase, Serva} | [57, 11, 2] |
| Additives (used during cell isolation) | {DNAse, HSA, none} | [19, 1, 50] |
| Performance (of the bioreactor culture) | {low, medium, high} | [18, 34, 18] |

### 2.3 t-Test and Wilcoxon Test

The 21 metric variables averaged over the groups of runs that were assigned to the different bioreactor performance levels were compared by the two-sided t-test and Wilcoxon's rank sum test (Wilcoxon-Mann-Withney test, MATLAB Statistics Toolbox, The MathWorks, Natick, MA, USA). Tests were performed comparing the groups 'high' versus 'low or medium', 'low' versus 'high or medium', 'low' versus 'high', 'high' versus 'medium' and 'low' versus 'medium'. The variables were ranked according to the *p*-values as determined by the t-test, i.e. according to the probability that two samples with a normal distribution of unknown but equal variances have the same mean.

### 2.4 Contingency Table Analysis

For each variable a 2 by 2 table was generated determining the numbers of runs assigned to two clusters with respect to the variable values as well as to the bioreactor performance. The clustering of the metric variables was performed using the minimum variance criterion. For the categorical variables the following pairs were analyzed: 'female' versus 'male', 'University of Wisconsin solution' versus 'histidine-tryptophane-ketoglutarate solution', 'Collagenase P' versus 'Liberase' and 'DNAse' versus 'no additives'. In respect of the bioreactor performance the pairs 'high' versus 'low or medium', 'low' versus 'high or medium', 'low' versus 'high', 'high' versus 'medium' and 'low' versus 'medium' were analyzed. For each 2 by 2 table the two-sided *p*-values were calculated by Fisher's exact test [7].

### 2.5 Random Forest Analysis

The variables were ranked according to their importance as calculated by Breiman's Random Forest algorithm [8, 9] available in R [10]. Before starting the algorithm *'randomForest'* missing values were imputed using the proximity obtained from the random forest imputing algorithm *'rfImpute'* configured for 5 iterations and 2500 trees. Running *'randomForest'* in the supervised mode an ensemble of 5000 trees was generated using a *mtry* parameter of 3 (estimated by *'tuneRF'* with stepfactor = 2, ntreeTry = 5000, improve = 0.05) and default values for the other parameters. The ensemble of the 5000 trees generated was then analyzed with respect to the first and second level split variables of the decision trees.

### 2.6 Support Vector Machines

The Support Vector Machine algorithm [11, 12] with a linear kernel and c = 0.1 (cost of constraint violation) together with a leave-one-out cross-validation was used in order to find single variables as well as pairs and triplets of variables that can robustly predict the bioreactor performance. This was done comparing the performance levels 'low', 'medium', 'high', 'low or medium' and 'high or medium'. When running the algorithm for missing values $x_{i,j}$, the corresponding runs $j$ were ignored when the variable $i$ was involved. The prediction accuracy was determined as the quotient $Q$ dividing the number of correctly predicted runs by the total number of tests (which equals the number of runs $J$ minus the number of runs ignored when dealing with missing values). $Q$ characterizes the predictive strength of the respective variable set. Single variables, pairs or triplets of variables with maximum prediction accuracy $Q$ were then selected.

## 3 Results and Discussion

Tables 3 and 4 show the results obtained by the ranking of the variables according to the *p*-values as calculated by the t-test and the exact Fisher's test as well as according to the importance as calculated by the Random Forest algorithm (see also Fig. 2). The t-test can only be applied to the metric variables. Comparing the performances 'low' versus 'high or medium' and 'low' versus 'medium', the 'Inoculated_Volume' was found to be significantly correlated to the bioreactor performance ($p<0.002$, see also

Fig. 1) by all three statistical tests (t-test, Wilcoxon's rank sum test, Table 3; exact Fisher's test for the contingency table analysis, Table 4).

The three statistical tests applied assess the relevance of individual variables but not of their combinations. To test such combinations of variables, Random Forests (RF) and Support Vector Machines (SVM) were applied.

The RF algorithm [8, 9] combines two powerful concepts in machine learning: bagging and random feature selection. Bagging stands for bootstrap aggregating which uses resampling to produce pseudo-replicates in order to improve predictive accuracy. Random feature selection can considerably improve predictive accuracy, too. Fig. 2 shows the variables ranked by their importance as calculated by the RF algorithm. The RF out-of-bag (OOB) estimate of error rate obtained was 44 %.

Each binary decision tree generated by the RF algorithm contains one first level split variable and two second level split variables (or one or two leaf nodes instead of them). Looking at individual variables, the 'Inoculated_Volume' most frequently occurred as first level and as second level split variable, i.e. in 10 % and 7 % of the cases, respectively (508 times in the 5000 first level nodes, 671 times in the 10000 second level nodes; Table 5). Often, one of the two second level split variables does not appear, i.e. there exists a leaf node following the first split. There are 1054 trees among the 5000 generated ones (21 %) with a leaf node instead of one of the two second level split variables (Table 5). These 1054 leaf nodes at the second level stand 430 times for 'low', 155 times for 'medium' and 469 times for 'high' performance.

**Table 3.** Rankings of the metric variables with respect to their influence on the bioreactor performance as obtained by the two-sided t-test for the performances 'high' versus 'low or medium' (A), 'low' versus 'high or medium' (B), 'low' versus 'high' (C), 'high' versus 'medium' (D) and 'low' versus 'medium' (E), respectively (*: $p<0.05$, **: $p=0.0013$, ***: $p=0.0003$); significant results obtained by Wilcoxon's rank sum test are indicated by crosses (+: $p<0.05$, ++: $p=0.0046$, +++: $p<0.001$)

| Variable | A<br>HvsL\|M | B<br>LvsH\|M | C<br>LvsH | D<br>HvsM | E<br>LvsM |
|---|---|---|---|---|---|
| BMI | *, + 1 | 6 | * 3 | 3 | 15 |
| Weight | 16 | 2 | 6 | 21 | 4 |
| Height | 7 | 10 | 17 | 2 | 3 |
| Age | + 3 | 4 | 4 | 6 | 9 |
| GGT | 9 | 20 | 14 | 10 | 16 |
| LDH | 18 | 13 | 21 | 14 | 7 |
| ALT | 17 | 12 | 18 | 15 | 8 |
| AST | 13 | 16 | 15 | 13 | 13 |
| DeRitis | 8 | 19 | 11 | 7 | 12 |
| Bilirubin | 11 | 11 | 7 | 12 | 17 |
| Urea | 5 | 5 | 19 | *, + 1 | *, + 2 |
| Preservation_Time | 14 | 14 | 13 | 16 | 19 |
| Organ_Weight | 19 | 8 | 12 | 19 | 5 |
| LDH_PS | 10 | 7 | 8 | 8 | 10 |
| AST_PS | + 12 | 3 | 9 | + 9 | 6 |
| GLDH_PS | 21 | 18 | 20 | 20 | 21 |
| AP_PS | 20 | 15 | 16 | 18 | 20 |
| Remaining_Mass | 6 | 9 | + 5 | 11 | 11 |
| Dissolved_Mass | * 2 | 17 | * 2 | 4 | 18 |
| Viability | 4 | 21 | 10 | 5 | 14 |
| Inoculated_Volume | 15 | ***, +++ 1 | *, ++ 1 | 17 | **, +++ 1 |

Searching for pairs of variables that most frequently appear in the set of generated decision trees, 'Urea' and 'Inoculated_Volume' were most often found jointly as first and second level split variables in 67 of the 5000 trees generated (1.3 %; Table 6). The occurrence of other pairs is less frequent, i.e. smaller than 1.3 % (Table 6). The 'pair' consisting of the variable 'Inoculated_Volume' (as the first level split variable) and a leaf node (instead of one of the two second level split variables) was found with the highest frequency, i.e. in 242 of the 5000 trees (5%; Table 6). Almost all, i.e. 239 of these 242 trees (99 %) can be expressed by the following rule:

IF *'Inoculated_Volume is smaller than B1',* THEN *'Performance is low'*. (1)

The split value *B1* is different for the 239 trees. The distribution of the split values *B1* is bimodal (Fig. 3): The split value *B1* for the 'Inoculated_Volume' lies 166 times (i.e. 69 %) between 350 and 380 mL and 52 times (22 %) between 250 and 260 mL. 21 times the split values *B1* lie outside these intervals.

**Table 4.** Rankings of the metric and the categorical variables with respect to their influence on the bioreactor performance as obtained by the two-sided exact Fisher's test of the contingency table analysis for the performances 'high' versus 'low or medium' (A), 'low' versus 'high or medium' (B), 'low' versus 'high' (C), 'high' versus 'medium' (D) and 'low' versus 'medium' (E), respectively (*: $p<0.05$, **: $p<0.002$) as well as the rankings according to the importance as calculated by the Random Forest algorithm (see also Fig. 2)

| Variable | A HvsL\|M | B LvsH\|M | C LvsH | D HvsM | E LvsM | F RF |
|---|---|---|---|---|---|---|
| BMI | 7 | 16 | 8 | 7 | 20 | 14 |
| Weight | 16 | 4 | 9 | 21 | 4 | 21 |
| Height | 12 | 22 | 16 | 11 | 19 | 17 |
| Age | * 2 | 12 | 5 | * 2 | 21 | 3 |
| GGT | 20 | 14 | 19 | 14 | 9 | 13 |
| LDH | 18 | 11 | 20 | 12 | 8 | 10 |
| ALT | 21 | 23 | 21 | 22 | 22 | 15 |
| AST | 22 | 24 | 22 | 23 | 23 | 6 |
| DeRitis | 8 | 15 | 7 | 8 | 16 | 8 |
| Bilirubin | 11 | 6 | 10 | 17 | 13 | 19 |
| Urea | 6 | 10 | 23 | 3 | 3 | 2 |
| Preservation_Time | 13 | 25 | 15 | 13 | 24 | 16 |
| Organ_Weight | 23 | 19 | 24 | 24 | 15 | 9 |
| LDH_PS | 15 | 8 | 11 | 20 | 11 | 7 |
| AST_PS | 24 | 13 | 13 | 25 | 10 | 4 |
| GLDH_PS | 19 | 18 | 25 | 15 | 12 | 11 |
| AP_PS | 17 | 20 | 17 | 18 | 25 | 20 |
| Remaining_Mass | 5 | 3 | 4 | 9 | 6 | 5 |
| Dissolved_Mass | * 1 | 7 | * 3 | * 1 | 18 | 12 |
| Viability | 10 | 21 | 14 | 6 | 14 | 18 |
| Inoculated_Volume | 14 | ** 1 | * 2 | 19 | ** 1 | 1 |
| Gender | 9 | 17 | 18 | 5 | 7 | 23 |
| Preservation_Solution | 25 | 5 | 12 | 16 | 5 | 24 |
| Digestion_Enzyme | 3 | 9 | 6 | 4 | 17 | 22 |
| Additives | 4 | * 2 | * 1 | 10 | 2 | 25 |

Searching for triplets of variables, those trees were most frequently found that have 'AST' as first level split variable with either 'Inoculated_Volume' (31 trees;

0.6 %) or 'Urea' (24 trees; 0.5 %) as one of the two second level split variables and a leaf node instead of the other second level split variable (Table 7).

While the RF method is based on the induction of decision trees with conditions *'variable value x < split value B'* that define regions with axis-parallel borders, SVM allow to generate classifiers with discriminating borders that are not restricted to be parallel to the axes. Using SVM it was searched for individual variables as well as pairs and triplets of variables that provide the highest prediction accuracy $Q$ as determined by leave-one-out cross-validation.

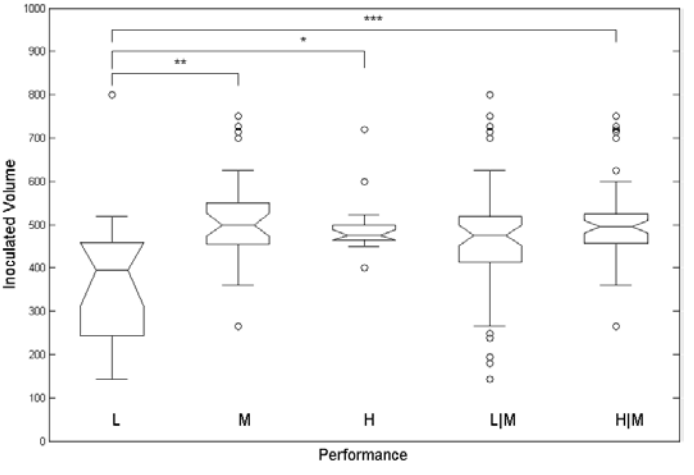


**Fig. 1.** Box plot of the values of the variable 'Inoculated_Volume'; each box shows the median, the lower and upper quartiles, the whiskers (length: 1.5-fold interquartile range) and the outliers (°, the rest of the data lies outside the whiskers); significant differences determined by the t-test and Wilcoxon's rank sum test are indicated by asterisks (*: $p$=0.016 and 0.0046, **: $p$=0.0013 and 0.0009, ***: $p$=0.0003 and 0.0005, respectively)

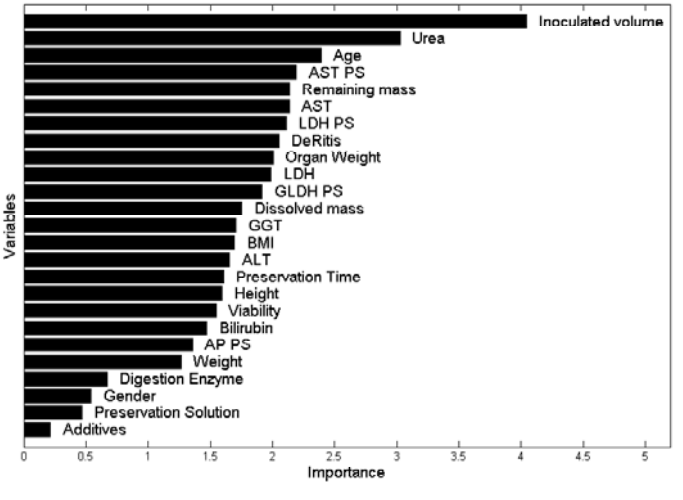


**Fig. 2.** Variable importance as calculated by the Random Forest algorithm [8, 9]

**Table 5.** Individual variables most frequently found as 1st or 2nd level split variable in the set of 5000 trees generated by the Random Forest algorithm; *N1, N2* – number of trees in which the variable represented the 1st level split variable or one of the two 2nd level split variables

| 1st Level Split Variable | *N1* | 2nd Level Split Variable | *N2* |
|---|---|---|---|
| Inoculated_Volume | 508 | - (Leaf node) | 1054 |
| Urea | 415 | Inoculated_Volume | 671 |
| AST | 319 | AST_PS | 516 |
| Age | 302 | Age | 486 |
| AST_PS | 298 | Urea | 485 |

**Table 6.** Pairs of variables most frequently found as 1st and 2nd level split variables in the set of 5000 trees generated by the Random Forest algorithm; *N12* – number of trees in which the pair represented the 1st level split variable and one of the two 2nd level split variables

| 1st Level Split Variable | 2nd Level Split Variable | *N12* |
|---|---|---|
| Inoculated_Volume | - (Leaf node) | 242 |
| AST | - (Leaf node) | 217 |
| LDH | - (Leaf node) | 86 |
| Dissolved_Mass | - (Leaf node) | 83 |
| Urea | Inoculated_Volume | 67 |
| Inoculated_Volume | AST | 64 |
| Urea | DeRitis | 63 |
| Urea | AST_PS | 55 |
| AST | Inoculated_Volume | 52 |
| Remaining_Mass | Inoculated_Volume | 51 |

**Table 7.** Triplets of variables most frequently found as 1st and 2nd level split variables in the set of 5000 trees generated by the Random Forest algorithm; *N122* – number of trees in which the triplet represented the 1st level split variable and both 2nd level split variables

| 1st Level Split Variable | 2nd Level Split Variables | | *N122* |
|---|---|---|---|
| AST | - (Leaf node), | Inoculated_Volume | 31 |
| AST | - (Leaf node), | Urea | 24 |
| Inoculated_Volume | - (Leaf node), | Dissolved_Mass | 17 |
| AST | - (Leaf node), | Height | 17 |
| Inoculated_Volume | - (Leaf node), | AST_PS | 16 |

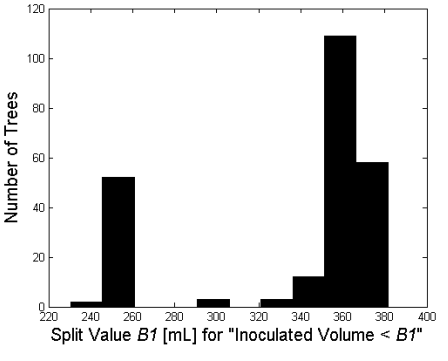


**Fig. 3.** Histogram of the split values *B1* in the condition of decision rule (1) as obtained by the Random Forest algorithm [8, 9]; (number of trees out of the 5000 generated versus split value)

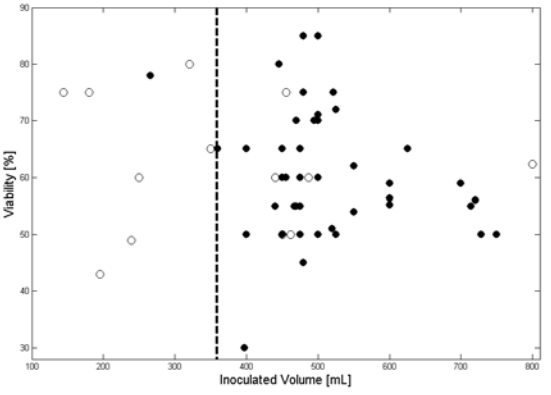


**Fig. 4.** 'Low' (o) versus 'high or medium' (•) bioreactor performance can be satisfactorily predicted by the variable 'Inoculated_Volume' alone; using the variable 'Viability' in addition does not improve the prediction accuracy considerably as was shown using SVM

Using SVM to distinguish between the performances 'low' and 'high or medium' yielded the highest values $Q$ for the individual variable 'Inoculated_Volume' ($Q$=0.836), for the variable pair 'Inoculated_Volume'/'Viability' ($Q$=0.844; Fig. 4) and for the variable triplet 'Inoculated_Volume'/'Viability'/'DeRitis' ($Q$=0.900). The value $Q$ was compared to the accuracy $Q_0$ of a dummy prediction 'all values are high or medium'. With a total number of 70 bioreactor runs and 18 low performance ones, $Q_0$ equals 0.74 (=1-18/70). However, neglecting runs with missing values, $Q_0$ varies: 0.74 for 'Inoculated_Volume', 0.78 for the pair 'Inoculated_Volume'/'Viability' and 0.77 for the triplet 'Inoculated_Volume'/'Viability'/'DeRitis'. Judged by the ratio $Q/Q_0$, the improvement in prediction accuracy by combining the 'Inoculated_Volume' as most important variable with other variables is rather small.

## 4 Conclusion

The variability of the performance (as single output) of 70 human liver cell bioreactor runs was studied based on 25 variables (as multiple inputs) that characterize donor organ properties, organ preservation, cell isolation and inoculation prior to bioreactor operation. The input-output relation was analyzed by various methods, in particular statistical tests (t-test, Wilcoxon's rank sum test, exact Fisher's test), Random Forests (RF, [8, 9]) and Support Vector Machines (SVM, [11, 12]) with a linear kernel as described in this paper. In addition, further methods were applied that yielded quite similar results which are not presented here: multivariate analysis by Principal Component Analysis (PCA), Independent Component Analysis (ICA) and Correspondence Analysis (CA), generation of classifiers by Induction of Decision Trees (See5, [13], with boosting and leave-one-out cross-validation), Support Vector Machines with polynomial and radial kernels, Recursive Partitioning, k-Nearest-Neighbor Classifiers, Naive Bayes Classifiers as well as cluster based rule generation [14] after clustering of the runs for the metric variables by the fuzzy c-means algorithm and minimum variance analysis with two clusters or an optimized cluster number (using 12 criteria).

The ‘Inoculated_Volume’ was found by all the applied methods as most important variable for the prediction of the liver cell bioreactor performance, in particular to predict ‘low’ performance. It is often followed next by ‘Urea’ (as shown in Table 3 for the t-test and Wilcoxon’s rank sum test as well as in Fig. 2 and Tables 4 to 7 for RF).

No robust results were obtained combining two or three variables. These more complex classifiers depend on the chosen method for classifier construction as well as on the selected data subset. In particular, only a maximum of 242 (5 %) out of the 5000 trees generated by Breiman’s RF algorithm were found to have the first level split variable, i.e. the ‘Inoculated_Volume’, and a leaf node (instead of one of the two second level split variables) in common (non-genuine variable pair; genuine pairs are even far less frequent; see Table 6). The split value *B1* for the first level split variable ‘Inoculated_Volume’ has a bimodal distribution between 230.5 and 381.5 mL (Fig. 3). In addition, just 31 or less (0.6 % or less) out of the 5000 trees generated by the RF algorithm have the first level split variable, one of the two second level split variables and a leaf node (instead of the other second level split variable) in common (non-genuine variable triplet; genuine triplets are almost not existent; see Table 7).

This unsatisfactory robustness of classifiers combining two or three of the variables also showed when SVM were employed. The ‘Inoculated_Volume’ on its own again proved to be the most discriminating variable to predict bioreactor performance, here with an accuracy of 84%. The inclusion of other variables, however, does not improve prediction accuracy considerably (see Fig. 4).

The positive correlation of the inoculated volume of cells with the bioreactor performance shown by different methods indicates that an improved performance can be achieved by increasing the cell volume that is inoculated into the bioreactor. Based on this theoretical result, it has to be established experimentally which maximum inoculation volume is practically feasible and which bioreactor performance can actually be achieved by this under real operating conditions.

Due to the widely consistent picture that evolved from the study using different methods (with the ‘Inoculated_Volume’ as the by far most important single variable for bioreactor performance prediction prior to operation) it may be concluded with respect to the problem investigated that only limited further information can be gained from the 25 variables analyzed here.

Further work should therefore be directed towards the analysis of derived variables that for instance relate to more cell-specific than solely bioreactor-specific characteristics. Also, other variables not included so far for various reasons should be part of an extended analysis. This for instance applies to additional donor organ properties, such as the liver injury (e.g. steatosis, cirrhosis, fibrosis and others) that led to the exclusion of the organ from transplantation and to the use of its cells in the bioreactor culture. Due to the diversity of these injuries and still low case numbers for several of them, the variable was not included in the present study.

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