Biliary Bicarbonate, pH and Glucose Are Suitable Biomarkers of Biliary Viability During Normothermic Machine Perfusion of Human Donor Livers

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SUPPLEMENTAL DIGITAL CONTENT

Table S1. Optimal Biliary Biomarker Cut-off Values per Time Point of NMP Based on AUC-ROC Analyses.

Biomarker	Number of Cases*	Cut-off Value	Time of NMP	AUC-ROC	P - value	Sens	Spec	PPV	NPV	LR+ (95% CI)	LR- (95% CI)
Biliary Bicarbonate Concentration	*21*	*18 mmol/L*	*2.0 h*	*0.91*	*0.002*	*83%*	*89%*	*91% (61 – 98%)*	*80% (53 – 94%)*	*7.50 (1.16 – 48.43)*	*0.19 (0.05 – 0.68)*
	21	27 mmol/L	2.5 h	0.86	0.006	83%	78%	83% (59 –95%)	78% (49 –93%)	3.75 (1.08 – 13.07)	0.21 (0.06 – 0.80)
	21	26 mmol/L	3.0 h	0.83	0.012	75%	78%	82% (56 – 94%)	70% (45 –87%)	3.38 (0.95 – 11.96)	0.32 (0.11 – 0.91)
	21	32 mmol/L	3.5 h	0.81	0.019	83%	67%	77% (56 – 90%)	75% (44 – 92%)	2.50 (0.96 – 6.52)	0.25 (0.07 – 0.96)
	20	32 mmol/L	4.0 h	0.80	0.025	82%	67%	75% (53 – 89%)	75% (44 – 92%)	2.45 (0.94 – 6.44)	0.27 (0.07 – 1.04)
	19	31 mmol/L	4.5 h	0.79	0.034	80%	67%	73% (50 – 88%)	75% (44 – 92%)	2.40 (0.91 – 6.36)	0.30 (0.08 – 1.13)
	19	32 mmol/L	5.0 h	0.78	0.041	80%	67%	73% (50 – 88%)	75% (44 – 92%)	2.40 (0.91 – 6.36)	0.30 (0.08 – 1.13)
	19	32 mmol/L	5.5 h	0.77	0.050	80%	67%	73% (50 – 88%)	75% (44 – 92%)	2.40 (0.91 – 6.36)	0.30 (0.08 – 1.13)
	19	32 mmol/L	6.0 h	0.78	0.041	80%	67%	73% (50 – 88%)	75% (44 – 92%)	2.40 (0.91 – 6.36)	0.30 (0.08 – 1.13)
Biliary pH	21	7.32	2.0 h	0.75	0.051	75%	78%	82% (56 – 94%)	70% (45 – 87%)	3.38 (0.95 – 11.96)	0.32 (0.11 – 0.91)
	21	*7.48*	*2.5 h*	*0.81*	*0.019*	*75%*	*67%*	*75% (53 – 89%)*	*67% (40 – 86%)*	*2.25 (0.84 – 6.00)*	*0.38 (0.13 – 1.11)*
	21	7.45	3.0 h	0.81	0.017	75%	78%	82% (56 – 94%)	70% (45 – 87%)	3.38 (0.95 – 11.96)	0.32 (0.11 – 0.91)
	21	7.46	3.5 h	0.79	0.028	75%	78%	82% (56 – 94%)	70% (45 – 87%)	3.38 (0.95 – 11.96)	0.32 (0.11 – 0.91)
	20	7.51	4.0 h	0.80	0.025	91%	67%	77% (57 – 90%)	86% (47 – 98%)	2.73 (1.06 – 7.00)	0.14 (0.02 – 0.93)
	19	7.53	4.5 h	0.85	0.010	90%	67%	75% (54 – 89%)	86% (47 – 98%)	2.70 (1.05 – 6.96)	0.15 (0.02 – 1.02)
	19	7.50	5.0 h	0.79	0.034	90%	67%	75% (54 – 89%)	86% (47 – 98%)	2.70 (1.05 – 6.96)	0.15 (0.02 – 1.02)
	19	7.51	5.5 h	0.81	0.022	80%	67%	73% (50 – 88%)	75% (44 – 92%)	2.40 (0.91 – 6.36)	0.30 (0.08 – 1.13)
	19	7.51	6.0 h	0.77	0.045	80%	67%	73% (50 – 88%)	75% (44 – 92%)	2.40 (0.91 – 6.36)	0.30 (0.08 – 1.13)
Biliary Glucose Concentration	*21*	*16 mmol/L*	*2.0 h*	*0.82*	*0.013*	*92%*	*78%*	*85% (62 – 95%)*	*88% (51 – 98%)*	*4.12 (1.20 – 14.17)*	*0.11 (0.02 – 0.72)*
	21	27 mmol/L	2.5 h	0.77	0.039	67%	89%	89% (55 – 98%)	67% (47 – 82%)	6.00 (0.91 – 39.74)	0.38 (0.16 – 0.86)
	21	24 mmol/L	3.0 h	0.81	0.015	69%	88%	90% (58 – 98%)	64% (43 – 80%)	5.54 (0.85 – 35.90)	0.35 (0.15 – 0.83)
	22	23 mmol/L	3.5 h	0.81	0.015	69%	89%	90% (58 – 98%)	67% (46 – 82%)	6.23 (0.95 – 40.96)	0.35 (0.15 – 0.81)
	22	22 mmol/L	4.0 h	0.81	0.016	69%	89%	90% (58 – 98 %)	67% (46 – 82%)	6.23 (0.95 – 40.96)	0.35 (0.15 – 0.81)
	21	23 mmol/L	4.5 h	0.79	0.028	67%	100%	100%	69% (50 – 83%)	n.a.[†]	0.33 (0.15 – 0.74)
	20	21 mmol/L	5.0 h	0.82	0.015	73%	100%	100%	75% (53 – 89%)	n.a.	0.27 (0.10 – 0.72)
	19	20 mmol/L	5.5 h	0.80	0.027	70%	100%	100%	75% (54 – 89%)	n.a.	0.30 (0.12 – 0.77)
	19	19 mmol/L	6.0 h	0.80	0.027	70%	100%	100%	75% (54 – 89%)	n.a.	0.30 (0.12 – 0.77)

| Biomarker	Number of Cases	Cut-off Value	Time of NMP	AUC-ROC	P - value	Sens	Spec	PPV	NPV	LR+ (95% CI)	LR- (95% CI)
Bile/Perfusate Glucose Ratio	*21*	*0.67*	*2.0 h*	*0.82*	*0.013*	*83%*	*78%*	*83% (59 – 95%)*	*78% (49 – 93%)*	*3.75 (1.08 – 13.07)*	*0.21 (0.06 – 0.80)*
	21	0.70	2.5 h	0.71	0.110	83%	78%	83% (59 – 95%)	78% (49 – 93%)	3.75 (1.08 – 13.07)	0.21 (0.06 – 0.80)
	22	0.66	3.0 h	0.78	0.030	85%	78%	85% (61 – 95%)	78% (48 – 93%)	3.81 (1.10 – 13.21)	0.20 (0.05 – 0.74)
	22	0.70	3.5 h	0.77	0.035	85%	78%	85% (61 – 95%)	78% (48 – 93%)	3.81 (1.10 – 13.21)	0.20 (0.05 – 0.74)
	21	0.73	4.0 h	0.76	0.042	77%	78%	83% (59 – 95%)	70% (45 – 87%)	3.46 (0.98 – 12.18)	0.30 (0.10 – 0.85)
	20	0.72	4.5 h	0.77	0.039	75%	67%	75% (53 – 89%)	67% (40 – 86%)	2.25 (0.84 – 6.00)	0.38 (0.13 – 1.11)
	19	0.74	5.0 h	0.75	0.063	82%	78%	82% (56 – 94%)	78% (49 – 93%)	3.68 (1.05 – 12.90)	0.23 (0.06 – 0.86)
	18	0.78	5.5 h	0.75	0.066	70%	89%	88% (51 – 98%)	73% (50 – 88%)	6.30 (0.95 – 41.78)	0.34 (0.13 – 0.89)
	18	0.72	6.0 h	0.73	0.086	80%	78%	80% (53 – 93%)	78% (49 – 93%)	3.60 (1.02 – 12.70)	0.26 (0.07 – 0.93)
Delta Perfusate Bile Glucose Concentration	21	10.0 mmol/L	2.0 h	0.74	0.065	92%	67%	77% (57 – 90%)	86% (47 – 98%)	2.73 (1.06 – 7.00)	0.14 (0.02 – 0.93)
	21	8.3 mmol/L	2.5 h	0.71	0.102	92%	67%	77% (57 – 90%)	86% (47 – 98%)	2.73 (1.06 – 7.00)	0.14 (0.02 – 0.93)
	22	**2.3 mmol/L**	**3.0 h**	**0.79**	**0.023**	**69%**	**89%**	**79% (59 – 90%)**	**86% (46 – 98%)**	**2.75 (1.07 – 7.04)**	**0.13 (0.02 – 0.86)**
	22	2.6 mmol/L	3.5 h	0.76	0.042	69%	89%	82% (56 – 94%)	70% (45 – 87%)	3.38 (0.95 – 11.96)	0.32 (0.11 – 0.91)
	21	4.9 mmol/L	4.0 h	0.74	0.065	75%	78%	80% (53 – 93%)	70% (46 – 87%)	3.27 (0.91 – 11.71)	0.35 (0.13 – 0.98)
	20	4.8 mmol/L	4.5 h	0.74	0.074	73%	78%	78% (49 – 93%)	70% (46 – 86.%)	3.15 (0.87 – 11.42)	0.39 (0.14 – 1.06)
	19	4.3 mmol/L	5.0 h	0.74	0.079	70%	78%	75% (45 – 92%)	70% (46 – 86%)	3.00 (0.81 – 11.08)	0.43 (0.16 – 1.15)
	18	2.9 mmol/L	5.5 h	0.73	0.102	67%	89%	86% (47 – 98%)	73% (51 – 87%)	6.00 (0.89 – 40.31)	0.38 (0.14 – 0.97)
	18	2.5 mmol/L	6.0 h	0.73	0.102	67%	89%	86% (47 – 98%)	73% (51 – 87%)	6.00 (0.89 – 40.31)	0.38 (0.14 – 0.97)
Biliary LDH Concentration	*21*	*3689 U/L*	*2.0 h*	*0.83*	*0.014*	*69%*	*88%*	*90% (58 – 98%)*	*64% (43 – 81%)*	*5.54 (0.85 – 35.90)*	*0.35 (0.15 – 0.83)*
	22	4710 U/L	2.5 h	0.78	0.03	69%	78%	82% (55 – 94%)	64% (42 – 81 %)	3.12 (0.87 – 11.15)	0.40 (0.16 – 0.96)
	22	2393 U/L	3.0 h	0.79	0.025	69%	67%	75% (53 – 89%)	60% (37 – 79%)	2.08 (0.77 – 5.60)	0.46 (0.18 – 1.18)
	22	3696 U/L	3.5 h	0.79	0.021	69%	89%	90% (58 – 98%)	67% (46 – 82%)	6.23 (0.95 – 40.96)	0.35 (0.15 – 0.81)
	21	2749 U/L	4.0 h	0.72	0.088	67%	78%	80% (53 – 94%)	64% (42 – 81%)	3.00 (0.83 – 10.86)	0.43 (0.18 – 1.03)
	21	2818 U/L	4.5 h	0.74	0.065	67%	78%	80% (53 – 94%)	64% (42 – 81%)	3.00 (0.83 – 10.86)	0.43 (0.18 – 1.03)
	20	2210 U/L	5.0 h	0.73	0.087	64%	78%	78% (49 – 93%)	64% (43 – 80%)	2.86 (0.78 – 10.52)	0.47 (0.20 – 1.10)
	20	2614 U/L	5.5 h	0.75	0.063	64%	78%	78% (49 – 93%)	64% (43 – 80%)	2.86 (0.78 – 10.52)	0.47 (0.20 –1.10)
	20	2445 U/L	6.0 h	0.69	0.149	64%	89%	88% (51 – 98%)	67% (47 – 82%)	5.73 (0.86 – 38.34)	0.41 (90.18 – 0.92)

Bold and italic values are the chosen cut-off points based on the first bile sample with an AUCROC >0.80 and a P-value <0.05.

* Values do not add up to the total of 23 due to livers not producing bile at every time point, or occasional errors in reporting values by the ABL90 FLEX analyzer.

Abbreviations: AUC-ROC, area under the curve of the receiver operating characteristics curve; CI, confidence interval; LDH, lactate dehydrogenase; LR+ positive likelihood ratio; LR-, negative likelihood ratio; NMP, normothermic machine perfusion; n.a., not applicable; NPV, negative predictive value; PPV, positive predictive value; Sens, sensitivity; Spec, specificity.

Figure S1. (Cumulative) bile production did not correlate with biliary pH, bicarbonate, glucose, bile/perfusate ratio or LDH.
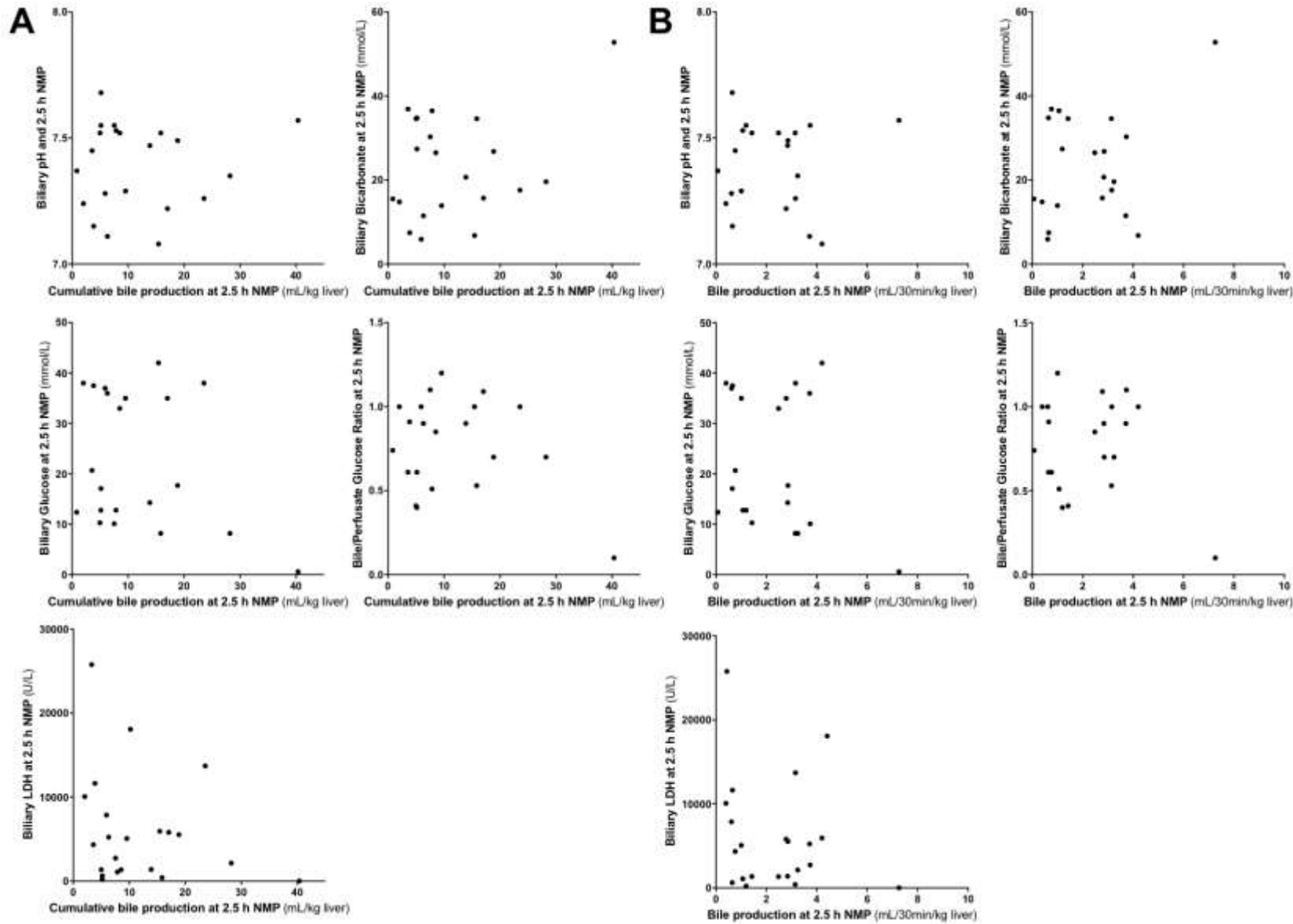
(A) Cumulative bile production and (B) bile production did not correlate with biliary pH, biliary bicarbonate, biliary glucose, bile/glucose perfusate ratio or biliary LDH.