Hematocrit: The Neglected Variable of Extracorporeal CO_2 Removal

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To the Editor:

Introduction

Carbon dioxide (CO_2), mainly carried in blood as bicarbonate, can be removed through the use of extracorporeal respiratory devices (1). The effects of several variables (*e.g.* gas flow and blood flow) on extracorporeal CO_2 removal ($ECCO_2R$) have been extensively explored. Less attention has been dedicated to the potential influence of hematocrit.

Methods

An *in-vitro* circuit primed with 3-L of swine blood was assembled with two membrane lungs (ML). The first ML, ventilated with CO_2, guaranteed a *constant* pCO_2 of 50 (tolerance of ±2 mmHg) at the inlet of the second ML, which removed CO_2 using a *fixed* oxygen flow of 5 L/min (**Figure 1A**). Blood temperature and flow through the second ML were constant (37°C and 250 ml/min).Blood was continuously dialyzed (MultiBic® 4mmol/L potassium, Fresenius) to keep lactate <2.5 mmol/L and to reach a baseline hematocrit of 35% (tolerance ±2%).

In addition, a filter was inserted before the second ML to *modulate* hematocrit by ultrafiltration. To *increase* hematocrit (by 10 and 20%) the ultrafiltrate was reinfused after the ML (Figure 1B); to *reduce* hematocrit (by 10 and 20%) the main blood flow was divided in a main branch, directed to the ML, and a secondary branch running through the filter. The obtained ultrafiltrate entered the main blood branch before the ML diluting blood. Blood of five pigs was tested twice (10 experiments). At each step (baseline, +10%, +20%, -10%, -20% of hematocrit), samples collected before (PRE) and after (POST) the second ML were analyzed.

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The amount of CO_2 removed (V_MCO_2) was measured, bicarbonate concentration ($[HCO_3^-]$), PRE and POST ML blood CO_2 content and strong ion difference (SID) were calculated (2, 3).

Statistical analysis

Data are presented as mean ± SD. Effects of Hct variation, PRE and POST ML effect and their interaction on variables were evaluated by generalized linear mixed models (fixed and random effects). Pairwise comparisons were tested with Bonferroni's correction. A polynomial maximum likelihood multilevel linear regression model was applied to evaluate the relationship between V_MCO_2 and hematocrit (2). A p-value<0.05 was considered statistically significant (SAS software v.9.4, SAS Institute, NC, USA and SigmaPlot, Systat Software, San Jose, CA).

Results

During the experiments, hematocrit ranged from 17±2% to 54±2% (**Table**). V_MCO_2 increased with hemoconcentration and was reduced with hemodilution (**Figure 1, Right**).

While PRE pCO_2 remained stable, POST pCO_2 was higher at steps with higher hematocrit (P<0.0001). Similarly, PRE $[HCO_3^-]$ remained relatively constant, while a progressive reduction according to hematocrit was observed in POST samples, justifying the increased CO_2 extraction. Given that red blood cells (RBCs) have lower CO_2 content than plasma (3), PRE CO_2 content was lower during hemoconcentration. Despite that, output CO_2 content was markedly reduced, resulting in enhanced CO_2 removal.

The changes in $[HCO_3^-]$ were closely mirrored by reductions in PRE-to-POST SID, attributed to increased chloride and decreased sodium concentrations in POST samples.

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PRE pH was relatively constant, while POST pH changed markedly with hematocrit, with very alkaline values observed during hemodilution, indicating a poor non-carbonic buffer power of samples with low hemoglobin concentration.

Discussion

Five variables regulating $ECCO_2R$ are classically mentioned: input pCO_2, gas flow, blood flow, blood temperature and ML size. Less attention has been dedicated to hematocrit (4). A recent *in-vitro* study evaluated the role of hematocrit on $ECCO_2R$ (5). However, a relatively narrow range of hematocrit was explored and the mechanisms of enhanced CO_2 removal were not investigated.

Let us therefore analyze the physiological mechanisms likely driving the observed increased $ECCO_2R$. Hypothesizing to use, in a setting akin to ours, a solution of water and electrolytes (Na^+=140 mEq/L, Cl^-=100 mEq/L, HCO_3^-=40 mEq/L), the only factor influencing $ECCO_2R$ would be dissolved CO_2, *i.e.* input pCO_2. In this context, bicarbonate, *i.e.* dissociated CO_2, can be considered as an "inert" form of CO_2 storage. Indeed, due to the absence of buffers, the reduction in $[HCO_3^-]$ induced by the pCO_2 decrease is negligible and, consequently, V_MCO_2 is limited. If albumin is added, V_MCO_2 will depend on the variation in pCO_2 (*as above*) and on the change in $[HCO_3^-]$. In this context, the amount of undissociated weak-non carbonic acids (AH), able to dissociate (A^-), is the driving factor allowing the $[HCO_3^-]$ reduction. If we add RBCs to the solution, these will allow greater electrolyte shifts and thus $[HCO_3^-]$ variations (2), effectively increasing V_MCO_2. In fact, RBCs act as internal acidifying elements, allowing the conversion of bicarbonate to pCO_2, driven by SID decreases (6, 7). The same concept of "bicarbonate displacement" was exploited in several experimental settings using exogenous acids (8, 9). This, however, lead to unwanted effects, such as

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persistent acid load or increased CO_2 production. In case of RBCs, the acid load results from charge redistribution, rather than its addition, and is therefore reversible.

The addition of RBCs (or their increased concentration) has another important consequence. As the CO_2 content of RBCs is lower than that of plasma (3), the net effect is a reduction of the amount of CO_2 reaching the ML. Interestingly, despite the reduced CO_2 content, the "exchangeable" CO_2 increases. We observed an increase in POST pCO_2 at the steps with higher hematocrit, suggesting that gas flow was insufficient for the achieved, higher POST pCO_2, driven by the higher conversion of bicarbonate. A simple implication is that an increase in gas flow (kept intentionally constant) would have further augmented $ECCO_2R$.

Should we therefore increase hematocrit in patients on $ECCO_2R$? A higher hematocrit optimizes both the delivery of oxygen and CO_2 removal. However, the increased viscosity of blood and the risk of transfusions may well outweigh any benefit and caution is required to extrapolate these results to the clinical arena (10).

The major limitation of our study is the variable concentration of plasma proteins. During hemoconcentration, we likely obtained also an increased albumin concentration. The observed slightly lower PRE pH and $[HCO_3^-]$ can be attributed to albumin's acidifying effect.

Conclusions

Hematocrit is a key variable in determining $ECCO_2R$ efficiency. RBCs enhance CO_2 removal, increasing exchangeable CO_2 and facilitating bicarbonate conversion to dissolved CO_2. Electrolyte shifts to and from RBCs guarantee electrical neutrality.

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Conflict of interest statement: Prof. Grasselli reports personal fees from Maquet, Draeger, Pfizer, Thermo Fisher, MSD and Gilead outside the submitted work. Prof. Alberto Zanella is inventor of patents related to the topic. The remaining authors have disclosed that they do not have any potential conflict of interest.

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Bibliography

1. Brusatori S, Zinnato C, Busana M, Romitti F, Gattarello S, Palumbo MM, et al. High- versus Low-Flow Extracorporeal Respiratory Support in Experimental Hypoxemic Acute Lung Injury. *American journal of respiratory and critical care medicine* 2023; 207: 1183-1193.

2. Langer T, Brusatori S, Carlesso E, Zadek F, Brambilla P, Ferraris Fusarini C, et al. Low noncarbonic buffer power amplifies acute respiratory acid-base disorders in patients with sepsis: an in vitro study. *Journal of applied physiology (Bethesda, Md : 1985)* 2021; 131: 464-473.

3. Siggaard-Andersen O WP, Fogh-Andersen N, Gøthgen IH. Measured and derived quantities with modern pH and blood gas equipment: Calculation algorithms with 54 equations. *Scandinavian Journal of Clinical and Laboratory Investigation* 1988; 48.

4. Bidani A, Crandall ED. Analysis of the effects of hematocrit on pulmonary CO2 transfer. *Journal of applied physiology: respiratory, environmental and exercise physiology* 1982; 53: 413-418.

5. May AG, Omecinski KS, Frankowski BJ, Federspiel WJ. Effect of Hematocrit on the CO2 Removal Rate of Artificial Lungs. *ASAIO journal (American Society for Artificial Internal Organs : 1992)* 2020; 66: 1161-1165.

6. Langer T, Scotti E, Carlesso E, Protti A, Zani L, Chierichetti M, et al. Electrolyte shifts across the artificial lung in patients on extracorporeal membrane oxygenation: interdependence between partial pressure of carbon dioxide and strong ion difference. *Journal of critical care* 2015; 30: 2-6.

7. Zadek F, Danieli A, Brusatori S, Giosa L, Krbec M, Antolini L, et al. Combining the physical-chemical approach with standard base excess to understand the compensation of

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respiratory acid-base derangements: an individual participant meta-analysis approach to data from multiple canine and human experiments. *Anesthesiology* 2023.

8. Cressoni M, Zanella A, Epp M, Corti I, Patroniti N, Kolobow T, et al. Decreasing pulmonary ventilation through bicarbonate ultrafiltration: an experimental study. *Critical care medicine* 2009; 37: 2612-2618.

9. Scaravilli V, Kreyer S, Belenkiy S, Linden K, Zanella A, Li Y, et al. Extracorporeal Carbon Dioxide Removal Enhanced by Lactic Acid Infusion in Spontaneously Breathing Conscious Sheep. *Anesthesiology* 2016; 124: 674-682.

10. Martucci G, Schmidt M, Agerstrand C, Tabatabai A, Tuzzolino F, Giani M, et al. Transfusion practice in patients receiving VV ECMO (PROTECMO): a prospective, multicentre, observational study. *The Lancet Respiratory medicine* 2023; 11: 245-255.

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TABLE 1: physiological variables

		TARGET Hct variation					
		-20	**-10**	**0 (Baseline)**	**+10**	**+20**	***P-value***
Hct (%)	**PRE**	17.0±2.3	26.4±3.1	35.4±1.9	44.7±1.8	53.9±2.4	<.0001
		†‡§#	‡§#	§#	#		
V_MCO_2 (mL/min)		31.0±4.8	37.3±5.7	42.5±6.3	44.9±6.7	48.7±7.6	<.0001
		†‡§#	‡§#	#	#		
pH	**PRE**	7.428±0.037	7.428±0.040	7.392±0.041	7.408±0.025	7.407±0.017	0.0064
		*‡	*‡	*	*	*	
	POST	8.012±0.043	7.924±0.051	7.810±0.066	7.761±0.057	7.721±0.037	<.0001
		†‡§#	‡§#	§#	#		
pCO_2 (mmHg)	**PRE**	50.5±1.4	49.5±1.8	50.3±1.0	49.6±0.7	50.2±1.0	0.1188
		*	*	*	*	*	
	POST	13.8±1.0	14.6±1.2	15.7±1.6	16.9±2.5	17.5±1.8	<.0001
		‡§#	§#	#			
Plasma [HCO_3^-] (mmol/L)	**PRE**	33.4±2.4	32.8±2.2	30.7±3	31.3±1.6	31.6±1.3	0.0015
		*‡§	*‡	*	*	*	
	POST	34.8±2.0	30.2±1.8	25.0±2.6	23.9±1.1	22.6±0.6	<.0001
		†‡§#	‡§#	#			
Plasma [TCO_2] (mmol/L)	**PRE**	35.0±2.3	34.3±2.2	32.3±3.0	32.9±1.6	33.1±1.3	0.0015
		‡§	*‡	*	*	*	
	POST	35.3±2.0	30.6±1.8	25.5±2.6	24.4±1.2	23.1±0.6	<.0001
		†‡§#	‡§#	#			
Blood [TCO_2] (mmol/L)	**PRE**	32.7±1.9	30.7±1.6	27.8±2.3	27.1±1.1	26.1±0.9	<.0001
		†‡§#	*‡§#	*	*	*	
	POST	32.8±1.8	27.3±1.5	21.7±2.1	19.8±0.9	17.8±0.6	<.0001
		†‡§#	‡§#	§#	#		
[Cl^-] (mEq/L)	**PRE**	109.1±2.5	109.4±2.8	109.1±3.2	109.3±2.8	109.1±3.0	0.8151
		*	*	*	*	*	
	POST	110.1±2.1	111.1±3.0	111.3±3.2	112±3.1	112.4±3.4	<.0001
		†‡§#	#	#			
[Na^+] (mEq/L)	**PRE**	141.8±1.8	141.7±1.8	141.2±2.0	141.5±1.8	142.5±1.4	0.1365
		*	*	*	*	*	
	POST	140.6±2.0	139.8±2.0	138.8±1.8	139.2±1.9	139.7±1.3	0.0125
		‡					
SID (mEq/L)	**PRE**	38.2±1.9	37.8±2.3	37±2.5	37.3±1.8	38.5±2.4	0.1329
		*	*	*	*	*	
	POST	35.8±1.6	34±2.3	32.2±2.3	32.1±1.9	32.3±2.8	<.0001
		‡§#	§				

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Mean ± SD of the physiological variables measured before (PRE) and after (POST) passing through the membrane lung at different target hematocrit variations. ***PRE and POST ML effect*** and ***interaction*** P-values (when applicable) were always <.0001; ***target Hct variation*** P-values were <.0001 except for [Cl^-] (P=0.0023), [Na^+] (P=0.0838) and SID (P=0.0013). P-values reported in Table represent: ***target Hct variation effect*** for "Hct" and "V_MCO_2", while, for all other variables ***target Hct variation effect sliced*** *for* ***PRE-POST*** values. Post-hoc analysis: * p<0.05 PRE vs POST; † p<0.05 vs Hct -10; ‡ p<0.05 vs Hct 0; § p<0.05 vs Hct +10; # p<0.05 vs Hct +20. Hct = hematocrit; V_MCO_2 = The amount of CO_2 removed by the membrane lung; pCO_2 = partial pressure of carbon dioxide; [HCO_3^-] = bicarbonate concentration; [TCO_2] = CO_2 content; [Cl^-] = chloride concentration; [Na^+] = sodium concentration; SID = strong ion difference.

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Figure Legends

Figure 1: Left: Scheme of the closed-loop circuit used for the experiments (**A**), describing both the system applied to achieve *hemoconcentration* (**B**) and *hemodilution* (**C**). The circuit was initially heparinized with 15000 Units of heparin. **Right**: The relationship between hematocrit and the amount of CO_2 removed through the membrane lung (V_MCO_2). Different symbols identify the 5 target Hct steps: baseline (black triangles), 10% Hct increase (white triangles), 20% Hct increase (black squares), 10% Hct decrease (white circles), 20% Hct decrease (black circles). Each experiment is identified by a different line. The polynomial maximum likelihood multilevel model results are represented by the blue line with 95% confidence interval (grey area). V_MCO_2 increased with square absolute Hct values according to the following model: $V_MCO_2 = 17.2 + 0.91 \times Hct - 0.0062 \times Hct^2$.

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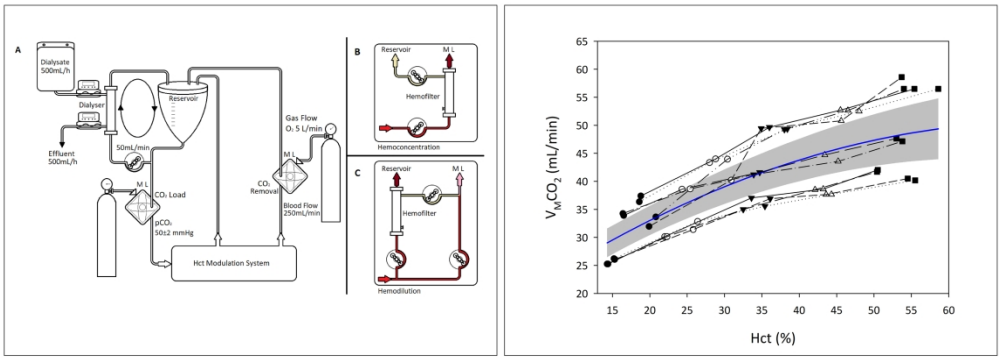

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284x108mm (300 x 300 DPI)

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