Diaspirin Cross-Linked Hemoglobin Infusion Did Not Influence Base Deficit and Lactic Acid Levels in Two Clinical Trials of Traumatic Hemorrhagic Shock Patient Resuscitation

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Background: Diaspirin cross-linked hemoglobin (DCLHb) has demonstrated a pressor effect that could adversely affect traumatic hemorrhagic shock patients through diminished perfusion to vital organs, causing base deficit (BD) and lactate abnormalities.

Methods: Data from two parallel, multicenter traumatic hemorrhagic shock clinical trials from 17 US Emergency Departments and 27 European Union prehospital services using DCLHb, a hemoglobin-based resuscitation fluid.

Results: In the 219 patients, the mean age was 37.3 years, 64% of the patients sustained a blunt injury, 48% received DCLHb resuscitation, and the overall 28-day mortality rate was 36.5%. BD data did not differ by treatment group (DCLHb vs. normal saline [NS]) at any time point. Study entry BD was higher in patients who died when compared with survivors in both studies (US: −14.7 vs. −9.3 and European Union: −11.1 vs. −4.1 mEq/L, $p < 0.003$) and at the first three time points after resuscitation. No differences in BD based on treatment group were observed in either those who survived or those who died from the hemorrhagic shock. US lactate data did not differ by treatment group (DCLHb vs. NS) at any time point. Study entry lactates were higher in US patients who ultimately died when compared with survivors (82.4 vs. 56.1 mmol/L, $p < 0.003$) and at all five postresuscitation time points. No lactate differences were observed between DCLHb and NS survivors or in those who died based on treatment group.

Conclusions: Although patients who died had more greatly altered perfusion than those who survived, DCLHb treatment of traumatic hemorrhagic shock patients was not associated with BD or lactate abnormalities that would indicate poor perfusion.

Key Words: Diaspirin cross-linked hemoglobin, Traumatic hemorrhagic shock, Base Deficit, Lactate, Resuscitation.

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Despite optimal resuscitation efforts, patients sustaining traumatic hemorrhagic shock have had an unacceptably high mortality rate. The search for a hemoglobin-based oxygen carrier (HBOC) that could be used as a resuscitation fluid both in the battlefield and civilian settings has as of yet not produced a solution that improves outcome.[1-3] Many of these solutions have demonstrated a pressor effect that is manifested by increased blood pressure (BP).[4-15]

The study of diaspirin cross-linked hemoglobin (DCLHb) in traumatic hemorrhagic shock patients included two parallel studies in the US Emergency Departments and in the European Union (EU) prehospital setting.[16,17] In the US study, DCLHb resuscitation was associated with increased mortality, and in the EU study, DCLHb was not associated with improved patient outcomes.[14] Resuscitation BPs from these two studies did not demonstrate a consistent DCLHb pressor effect.[18] Despite this lack of observed pressor effect, DCLHb and other HBOCs could have a deleterious effect on patient outcome if these solutions adversely alter perfusion to vital organs as manifested by a worsening of serum base deficits (BDs) and elevated lactate levels, both of which have been shown to correlate with morbidity and mortality in traumatic hemorrhagic shock.[19-33]

This study determined if DCLHb use in traumatic hemorrhagic shock resuscitation was correlated with BD and lactate (LA) abnormalities based either on treatment group (DCLHb vs. normal saline [NS]) or on patient outcome (28-day mortality). The presence or absence of any adverse perfusion effects based on treatment group will provide a better understanding if the suspected pressor effect of solutions such as DCLHb should limit future study of the HBOCs in the setting of uncompensated traumatic hemorrhagic shock.

METHODS

The clinical trials of DCLHb in traumatic hemorrhagic shock occurred between February 1997 and January 1998 in the US study, and from July 1997 to May 1998 in the EU study.[16,17] Because of an observed increased mortality in the DCLHb-treated patients in the US study, the study was terminated by the Data Safety Monitoring Board after the enrollment of 98 patients. At that time, the EU study was also halted and analysis of the EU data demonstrated no benefit with the use of DCLHb, resulting in the final termination of this EU study after the enrollment of 121 patients.

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The database for the current analysis of BDs and lactic acid levels after DCLHb use in traumatic hemorrhagic shock came from the original datasets that were collected by Baxter HealthCare for the US and EU studies. BD data were obtained at five-time points in both the US Emergency Department and EU prehospital studies and are reported in milliequivalent per liter. In the US trial, serum lactate levels were also obtained at five-time points and are reported in milligram per deciliter (mg/%). Data presented are mean values ± SD for all patients based on treatment group (DCLHb vs. NS) and 28-day outcome status (survived vs. expired).

TABLE 1. Patient Demographics and Clinical Variables in the US and EU DCLHb Clinical Trials

Age (yrs)	37.3 ± 17.2
Gender	
Male	159 (72.6)
Female	60 (27.4)
Study setting	
US	98 (44.7)
EU	121 (55.3)
Resuscitation fluid	
DCLHb	106 (48.4)
NS	113 (51.6)
Injury mechanism	
Blunt	139 (63.5)
Penetrating	80 (36.5)
Blunt injury type	
Motor vehicle crash	94 (67.6)
Fall	32 (23.0)
Others	13 (9.4)
Penetrating injury type	
Gun shot wound	35 (43.8)
Stab wound	27 (33.8)
Others	11 (13.8)
Motor vehicle crash	6 (7.5)
Fall	1 (1.3)
Injury severity score	30.4 ± 18.1
28-d outcome	
Survived	139 (63.5)
Died	80 (36.5)

The values given in the parentheses represent percentage.

The statistical analysis of the BD and lactic acid data included the comparisons of mean and SD data and the distributions of these two perfusion markers. Regression analysis was used to test the association among BD, lactate, the use of DCLHb, and other demographic and clinical variables at each of the five-time points.

The protocols used in US and EU clinical trials were approved by the institutional review board of each participating institution before the enrollment of any subjects. Trials were conducted in compliance with all regulations for good clinical trials and practice. The US study was conducted under federal regulations governing emergency research with an exception to informed consent. The current analysis of the data was conducted with institutional review board approval from the University of Illinois at Chicago.

RESULTS

There were a total of 219 patients studied, with 45% coming from the US Emergency Department study (Table 1). The mean age was 37.3 years, 64% of the patients sustained a blunt injury, 48% received DCLHb resuscitation, and the overall 28-day mortality rate was 36.5%.

When analyzed by survival status in the combined US and EU dataset, BD was significantly greater in patients who died when compared with those who survived at the first four-time points: BD 1 (−13.0 vs. −7.0 mEq/L, $p = 0.001$), BD 2 (−9.7 vs. −4.2 mEq/L, $p = 0.005$), BD 3 (−8.5 vs.

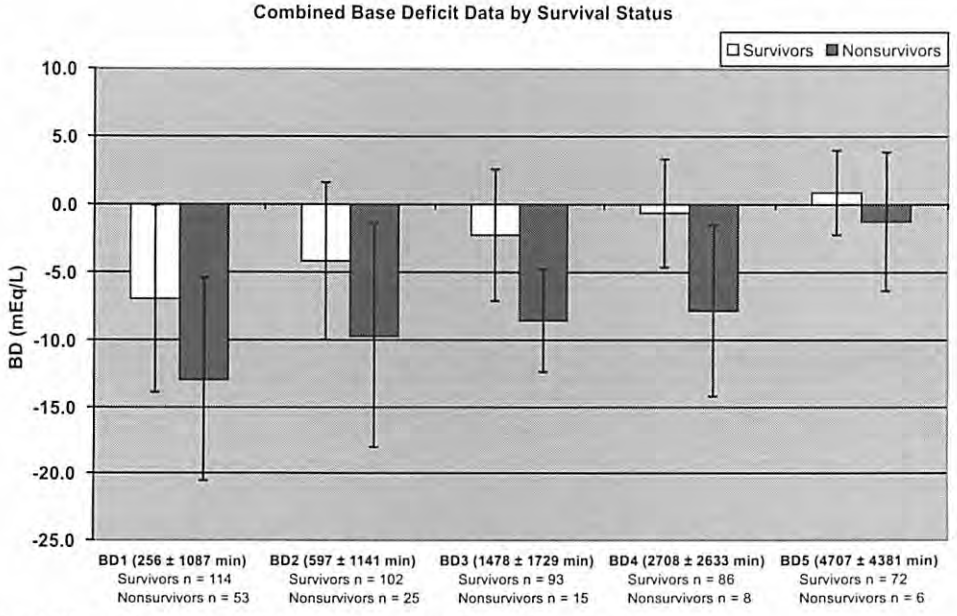

Figure 1. Combined US and EU base deficit data based on survival status.

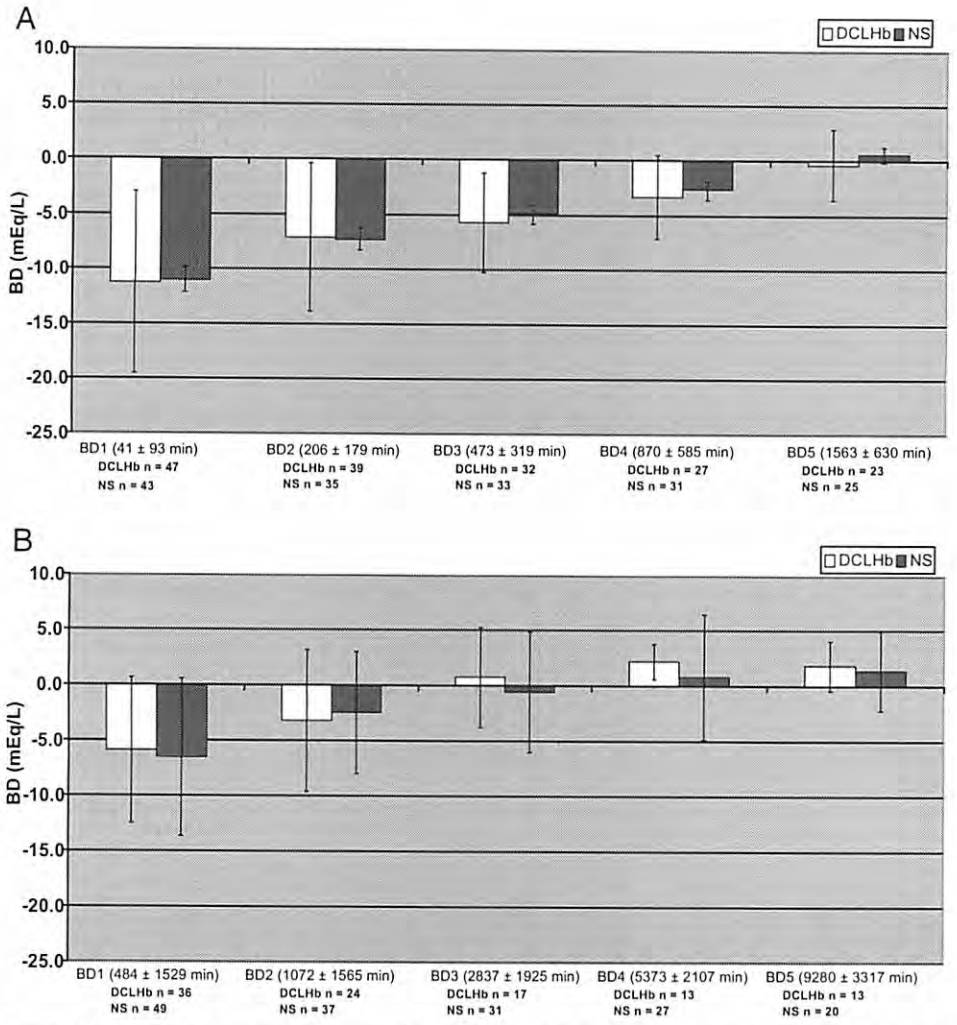

Figure 2. (*A*) US base deficit data by treatment group. (*B*) EU base deficit data by treatment group. NS, normal saline.

−2.3 mEq/L, $p = 0.001$), and BD 4 (−7.8 vs. −0.6 mEq/L, $p = 0.016$; Fig. 1).[17]

In the US clinical trial, BD did not differ by treatment group at any time point (Fig. 2, A). No differences in BD based on treatment group were observed in either those who survived or those who died from the hemorrhagic shock (Fig. 3, A and B, Table 2).

When stratifying the US BD data into four ranges (>0, 0 to −4.9, −5.0 to −9.9, and −10 mEq/L or worse) and into dichotomous BD ranges (<−4.9 vs. −5 mEq/L or worse), there were no BD differences at any of the five-time points based on treatment group (Table 3). The overall BD distributions were noted to be different based on 28-day survival status at the first, third, and fourth time points. The distributions were also noted to be different based on survival status when dichotomized to <−4.9 versus −5 mEq/L or worse at the first and third time points.

In the EU prehospital clinical trial, BD again did not differ by treatment group at any time point (Fig. 2, B). No differences in BD based on treatment group were observed in either those who survived or those who died from the hemorrhagic shock (Fig. 4, A and B, Table 4).

When stratifying the EU BD data into four ranges (>0, 0 to −4.9, −5.0 to −9.9, and −10 mEq/L or worse) and into dichotomous BD ranges (<−4.9 vs. −5 mEq/L or worse), there were no BD differences at any of the five-time points based on treatment group (Table 5). The overall BD distributions were noted to be different based on 28-day survival status at every time point. The distributions were also noted to be different based on survival status when dichotomized to <−4.9 versus −5 mEq/L or worse at the first, third, fourth, and fifth time points.

Lactate values in the US study were comparable in the two treatment groups in all but the second time point, which occurred at an average of 519 minutes after the infusion of DCLHb or the NS control solution (Fig. 5, A), When analyzed by survival status, lactates were significantly greater in patients who died when compared with those who survived at all of the time points: LA 1 (82.4 vs. 56.1 mg/dL, $p = 0.003$), LA 2 (73.8 vs. 33.5 mg/dL, $p = 0.004$), LA 3 (68.1 vs. 28.2

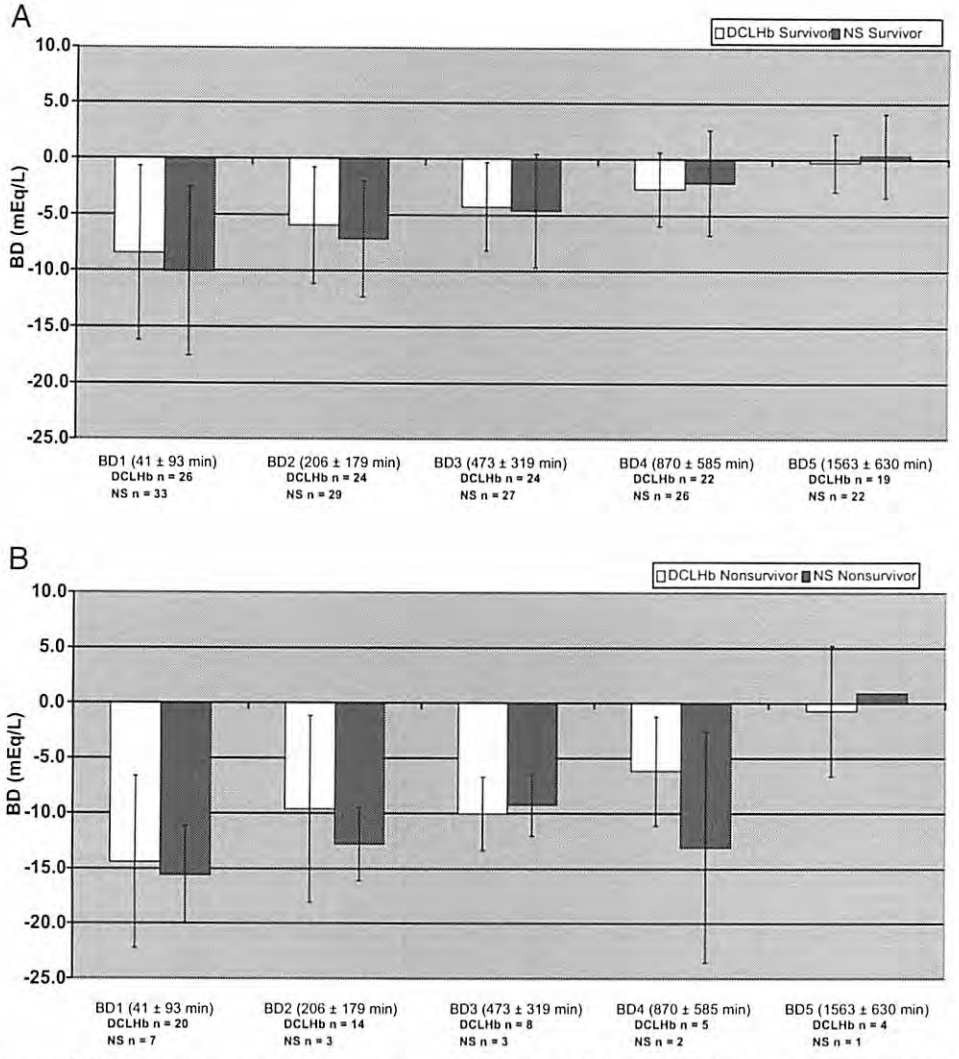

Figure 3. (*A*) US base deficit data by treatment group in survivors. (*B*) US base deficit data by treatment in nonsurvivors. NS, normal saline.

TABLE 2. US DCLHb Clinical Trial Base Deficit Data Based on Treatment Group and Survival Status (mEq/L)

Time Point	DCLHb	NS	*p*
Survival status: survivors			
BD 1	−8.4 ± 7.8	−10.1 ± 7.5	ns
BD 2	−6.0 ± 5.2	−7.2 ± 5.2	ns
BD 3	−4.3 ± 3.9	−4.6 ± 5.1	ns
BD 4	−2.7 ± 3.3	−2.1 ± 4.7	ns
BD 4	−0.3 ± 2.6	0.3 ± 3.7	ns
Survival status: nonsurvivors			
BD 1	−14.4 ± 7.8	−15.6 ± 4.4	ns
BD 2	−9.6 ± 8.5	−12.8 ± 3.3	ns
BD 3	−10.1 ± 3.3	−9.2 ± 2.8	ns
BD 4	−6.2 ± 4.9	−13.1 ± 10.5	ns
BD 5	−0.7 ± 5.9	−0.9 ± 0.0	ns

NS, normal saline; ns, not significant.

mg/dL, $p \leq 0.001$), LA 4 (83.7 vs. 24.1 mg/dL, $p = 0.001$), and LA 5 (33.7 vs. 18.1, mg/dL, $p = 0.03$; Fig. 5, B). In the US study, lactate values did not differ based on survival status in both of the DCLHb and NS treatment groups (Fig. 6, A and B, Table 6).

When stratifying the US lactate data into five ranges (>90, 61–90, 31–60, 10–30, and <10 mg/dL) and into dichotomous lactate ranges (≥45 and <45 mg/dL), there were no differences at any of the five-time points based on treatment group. The overall lactate distributions were noted to be different based on 28-day survival status at each time point (Table 7). The distributions were also noted to be different based on survival status when dichotomized to ≥45 mg/dL and <45 mg/dL at the first, second, third, and fourth time points.

Regression analysis did not demonstrate any relationship between treatment group and the observed BD or lactate values from either study except at the second time point for lactate in the US study where DCLHb patients had a lactate

TABLE 3. US DCLHb Clinical Trial Base Deficit Distribution Data Based on Survival Status

	Base Deficit (mEq/L)				
Time Point	>0	0 to −4.9	−5 to −9.9	−10 or Worse	*p*
BD1					
Died	0 (0.0)	1 (3.7)	6 (22.2)	20 (74.1)	0.03
Survived	5 (8.5)	10 (16.9)	19 (32.2)	25 (42.4)	
BD2					
Died	1 (5.9)	1 (5.9)	7 (41.2)	8 (47.1)	0.13
Survived	4 (7.5)	15 (28.3)	22 (41.5)	12 (28.3)	
BD3					
Died	0 (0.0)	1 (9.1)	5 (45.5)	5 (45.5)	0.02
Survived	10 (19.6)	19 (37.3)	15 (29.4)	7 (13.7)	
BD4					
Died	0 (0.0)	3 (42.9)	1 (14.3)	3 (42.9)	0.01
Survived	15 (31.3)	22 (45.9)	9 (18.8)	2 (4.2)	
BD5					
Died	2 (40.0)	2 (40.0)	1 (20.0)	0 (0.0)	0.64
Survived	19 (46.3)	19 (46.3)	3 (7.3)	0 (0.0)	
	>0 to −4.9	−5 or worse	OR	95% CI	
BD1					
Died	1 (3.7)	26 (96.3)	4.43	1.1–190	0.035
Survived	15 (25.4)	44 (74.6)			
BD2					
Died	2 (11.8)	15 (88.2)	2.50	0.78–29.7	0.11
Survived	19 (35.8)	34 (64.2)			
BD3					
Died	1 (9.1)	10 (90.9)	6.47	1.51–296	0.011
Survived	29 (56.9)	22 (43.1)			
BD4					
Died	3 (42.9)	4 (57.1)	2.09	0.69–31.0	0.15
Survived	37 (77.1)	11 (22.9)			
BD5					
Died	4 (80.0)	1 (20.0)	0.01	0.00–55.4	0.91
Survived	38 (92.7)	3 (7.3)			

The values given in the parentheses represent percentage.
OR, odds ratio; CI, confidence interval.

level of 49 mg/dL ± 32 mg/dL and NS resuscitated patients had a lactate level of 34 mg/dL ± 24 mg/dL. Otherwise, the observed BD and lactate values were more likely to be related to clinically relevant variables such as Glasgow Coma Scale, Injury Severity Score, mechanism of injury, Revised Trauma Score, time to study entry, total amount of blood transfused, or the preinfusion Hb level.

DISCUSSION

Development of a HBOC that could improve traumatic hemorrhagic shock patient outcomes both in the civilian and military setting continues despite previous setbacks.[34-40] There is a continuing effort to develop a solution that can be carried by medics or paramedics that is stable at room temperature and could be easily used in a broad population of traumatic hemorrhagic shock patients. The development of such a solution has been hampered in part by concerns based on the DCLHb experience regarding the pressor effects of HBOCs, with the belief that these solutions could cause poor perfusion to vital organs.[7,15,41-46]

The use of DCLHb and other oxygen-carrying hemoglobin solutions with a pressor effect could possibly hinder successful patient resuscitation through impaired perfusion to vital organs and a worsening acid/base balance with worsening BDs and higher lactate levels.[2-31,47] These products could, as a result of an adverse pressor effect, alter perfusion to vital organs in a way that could cause the occurrence of multisystem organ failure after the acute resuscitation, which would then increase mortality during the first 28 days.[20,24-26,32,48-53] The suspected HBOC pressor effect could also complicate the resuscitation of traumatic hemorrhagic shock patients because systolic BP (SBP) elevations with HBOC use lead clinicians to under-resuscitate these patients, either with crystalloids or with blood, causing worsening perfusion over time due to inadequate intravascular volumes, again leading to worsening BDs and higher lactate concentrations.

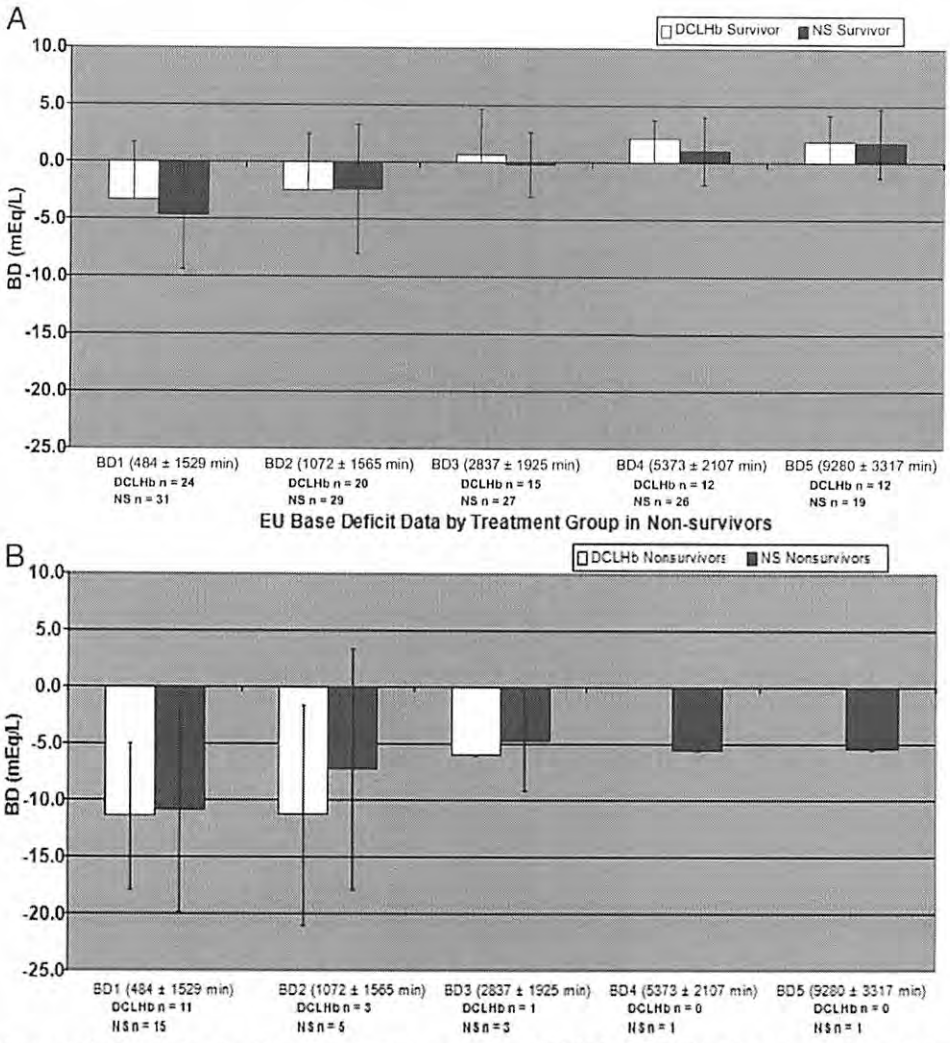

Figure 4. (*A*) EU base deficit data by treatment group in survivors. (*B*) EU base deficit data by treatment in nonsurvivors.

TABLE 4. EU DCLHb Clinical Trial Base Deficit Data Based on Treatment Group and Survival Status (mEq/L)

Time Point	DCLHb	NS	*p*
Survival status: survivors			
BD 1	−3.3 ± 5.0	−4.7 ± 4.7	NS
BD 2	−2.4 ± 4.9	−2.3 ± 5.6	NS
BD 3	0.7 ± 4.0	−0.2 ± 2.9	NS
BD 4	2.1 ± 1.6	1.1 ± 3.0	NS
BD 4	1.9 ± 2.3	1.7 ± 3.0	NS
Survival status: nonsurvivors			
BD 1	−11.5 ± 6.4	−10.9 ± 9.0	NS
BD 2	−11.3 ± 9.7	−7.3 ± 10.6	NS
BD 3	−6.0	−4.7 ± 4.5	NS
BD 4		−5.6	
BD 5		−5.5	

Although this is a post-hoc analysis from studies conducted over 10 years ago, these data are still important to further research into HBOCs because BD and lactate derangements are critical considerations during blood substitute clinical trials.[1,18] The lack of an observed BD or lactate effect in the DCLHb clinical trials supports further HBOC clinical trials that search to obtain a safe and efficacious product. The use of these two DCLHb studies as a model for perfusion effects is an important one, given that this pure tetrameric DCLHb solution was tested as a therapeutic because of its consistent pressor effect and purported improved perfusion to vital organs.[13,41,54] Although pressor effects are thought to be more consistently observed with DCLHb, a pure solution of hemoglobin tetramer, these pressor effects might also occur with other hemoglobin solutions, such as HBOC-201, PolyHeme, or Hemolink, such that the risk of a worsening acid/base balance must be studied first using the data from these two DCLHb traumatic hemorrhagic shock clinical trials.[5,30,42,55-61] HBOCs have exhibited pressor effects thought to be due to nitric oxide scavenging.[11,45,62] Nitric oxide is a key vasodilator in the bloodstream, which is synthesized by blood vessel wall endothelial cells and aids in stimulating the relaxation of nearby smooth muscle cells.[63] DCLHb is a pure tetrameric hemoglobin solution thought to have the greatest pressor effect of the clinically tested HBOCs. Other HBOCs

TABLE 5. EU DCLHb Clinical Trial Base Deficit Distribution Data Based on Survival Status

	Base Deficit (mEq/L)				
Time Point	>0	0 to −4.9	−5 to −9.9	−10 or Worse	*p*
BD1					
Died	2 (7.7)	3 (11.5)	6 (23.1)	15 (57.7)	0.001
Survived	8 (13.6)	29 (49.2)	18 (30.5)	4 (6.8)	
BD2					
Died	1 (12.5)	3 (37.5)	2 (25.0)	2 (25.0)	0.001
Survived	18 (34.0)	23 (43.4)	9 (17.0)	3 (5.7)	
BD3					
Died	0 (0.0)	2 (50.0)	2 (50.0)	0 (0.0)	0.004
Survived	23 (52.3)	19 (43.2)	2 (4.5)	0 (0.0)	
BD4					
Died	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0.001
Survived	29 (74.4)	9 (23.1)	1 (2.6)	0 (0.0)	
BD5					
Died	0 (0.0)	0 (0.0)	1 (100)	0 (0.0)	0.001
Survived	24 (75.0)	8 (25.0)	0 (0.0)	0 (0.0)	
	>0 to −4.9	−5 or worse	OR	95% CI	
BD1					
Died	5 (19.2)	21 (80.8)	11.97	2.10–25.2	0.001
Survived	37 (62.7)	22 (37.3)			
BD2					
Died	4 (50.0)	4 (50.0)	1.46	0.55–20.0	NS
Survived	41 (77.4)	12 (22.6)			
BD3					
Died	2 (50.0)	2 (50.0)	4.86	1.22–537	0.027
Survived	42 (95.5)	2 (4.5)			
BD4					
Died	0 (0.0)	1 (100)			0.037
Survived	38 (97.4)	1 (2.6)			
BD5					
Died	0 (0.0)	1 (100)			0.0054
Survived	32 (100)	0 (0.0)			

The values given in the parentheses represent percentage.
OR, odds ratio; CI, confidence interval.

such as PolyHeme, which are composed of mostly nontetrameric hemoglobin molecules, have been suggested to have less of a pressor effect. Despite different chemical modifications, such as synthesis with glutaraldehyde, *O*-raffinose, activated dextran, and molecular cross-linking, all HBOCs have been shown to exhibit some hemodynamic effects resulting from likely endothelial nitric oxide scavenging.[64]

Regardless of the differences of the hemoglobin solutions with regard to pressor effects, the absence of adverse pressor effects with DCLHb use suggests that all HBOCs may be safely used in the treatment of traumatic hemorrhagic shock patients, especially if these effects are thought to be related to the presence of pure tetrameric Hb.[12]

As has been seen previously, patients who ultimately died in these clinical trials had more derangements in their BDs and lactates, illustrating worsened perfusion.[10] The important question related to the use of HBOCs is whether any proposed pressor effect will be the cause of worsened perfusion, abnormal acid/base status, and higher mortality. In the BP effect analysis of DCLHb, patients who died did not exhibit elevated BPs.[18] Elevated BPs were actually more often observed in patients who survived. As such, there is no suggestion of a significant correlation between DCLHb-induced elevated BPs and worsened BDs and lactates, suggestive of poor perfusion. If there is no clinically measurable acid/base imbalance due to impaired perfusion with DCLHb use from these studies, it might be possible to infer that perfusion problems will also not be observed with the other hemoglobin solutions that are studied for use in the resuscitation of traumatic hemorrhagic shock patients.

The use of patient data from both the US and EU studies effectively balances the study of shock in penetrating and blunt trauma victims, as well as those who had minimal fluid resuscitation in the prehospital setting before DCLHb use (EU protocol) and those with the infusion of crystalloids by Emergency Medical Services (EMS) paramedics before

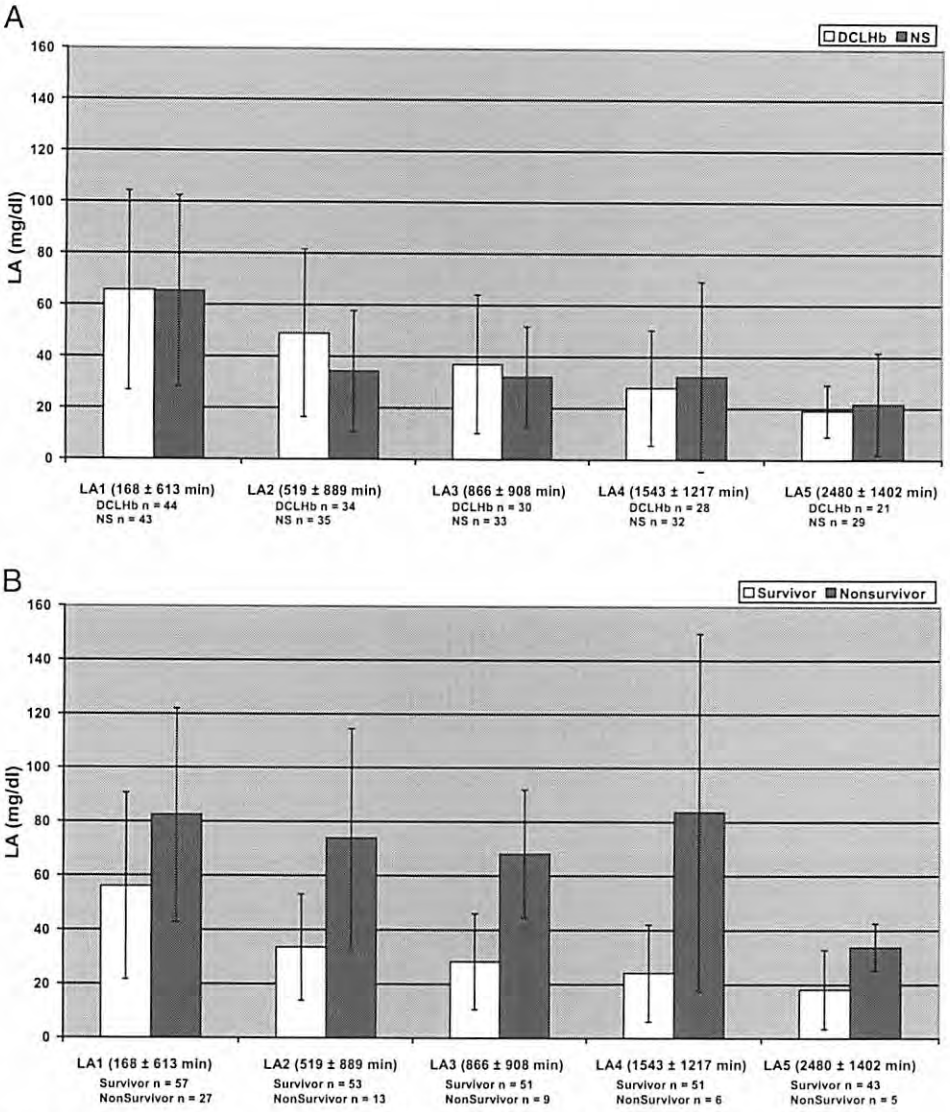

Figure 5. (*A*) US lactate data by treatment group. (*B*) US lactate data by survival status.

DCLHb use (US protocol).[13] The patients in this study are similar in mechanism of injury, injury severity, and mortality rate to trauma populations from other traumatic hemorrhagic shock studies.[65-67]

BD and lactate elevations were correlated with patient outcome in these trials, consistent with data that suggest that worse BDs are associated with higher mortality in traumatic hemorrhagic shock.[22,24,26,28,52,68-71] The data from these studies also confirm the mortality predicting power of BD and lactate levels in traumatic hemorrhagic shock patients.[6] As such, the potential adverse effect of DCLHb treatment on perfusion can be analyzed by measuring BDs and lactates during the shock resuscitation.

There were no differences in BD mean values or distributions both in the US and in the EU studies based on treatment group.[3] This suggests that there were no adverse perfusion effects to vital organs with the use of DCLHb, a finding that is consistent with the lack of observed BP effects from these two clinical trials.[18] The FDA concern that the vasoactivity and potential toxicity of HBOCs are the cause of worsened patient outcome due to impaired perfusion is not supported by the data from these two DCLHb clinical trials.[4] BD and lactate levels, which correlate with perfusion, demonstrated no difference based on treatment with DCLHb.[5] Freilich et al.,[72] in an orthopedic clinical trial, also found no difference in vasoactivity with a hemoglobin solution, HBOC-201, in young, stable trauma patients who experienced a hypotensive episode.

There were also no observed differences in the mean lactate or the distribution of lactates in the US study based on treatment group. This again suggests that there were no adverse perfusion effects to vital organs with the use of

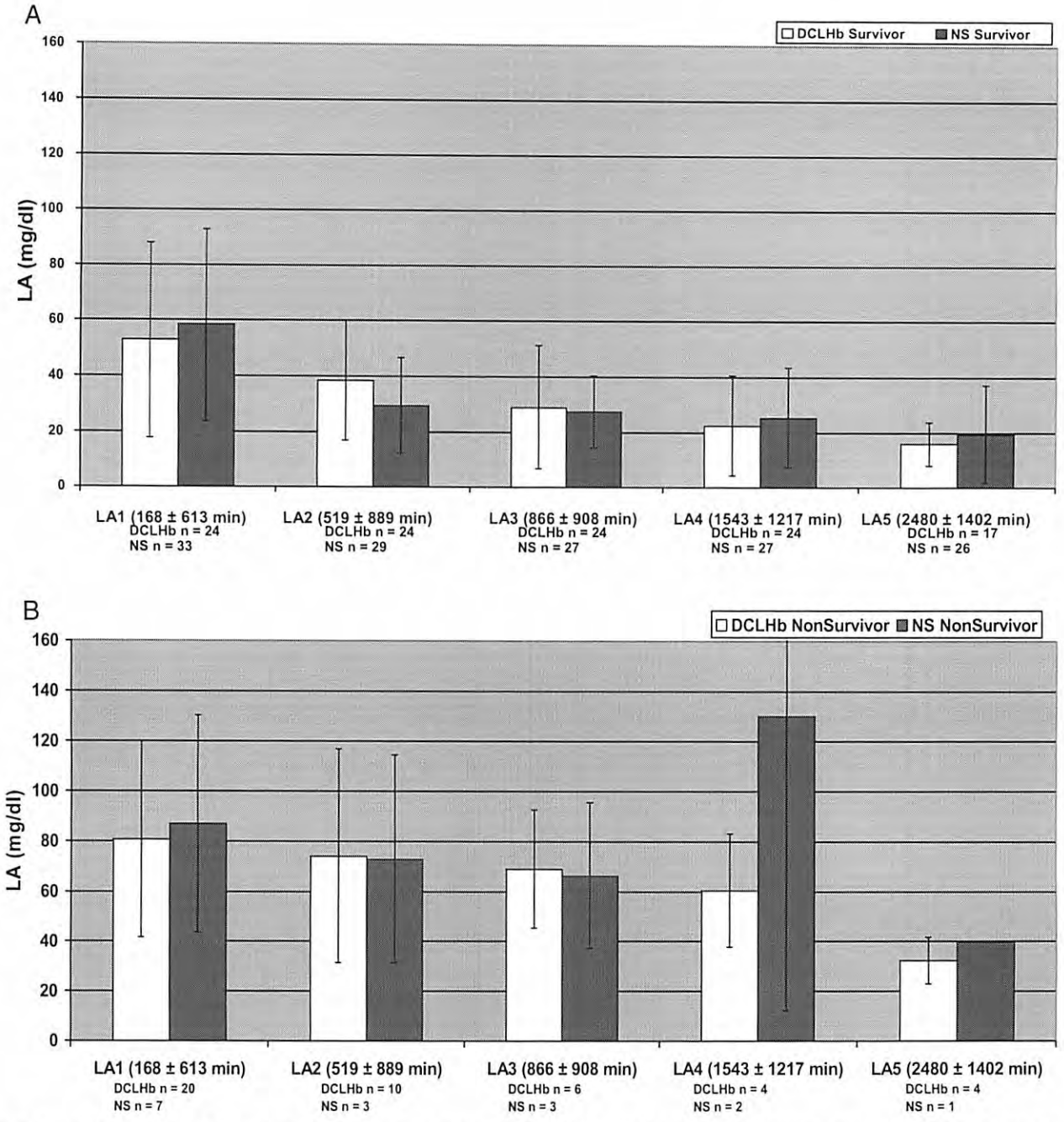

Figure 6. (*A*) US lactate data by treatment group in survivors. (*B*) US lactate data by treatment group in nonsurvivors. NS, normal saline.

DCLHb, even though mortality rates were higher in patients treated with DCLHb in this study.[16] As with the BD findings, there were lactate differences based on patient outcome, consistent with data that suggest that higher lactate levels are associated with higher mortality in traumatic hemorrhagic shock patients.[26,49,70,73-76]

Because HBOCs have been observed to reduce the measured lactate levels, it is possible that the lack of observed difference in lactates from the US study is due to this laboratory assay problem causing all lactate levels to be reduced and the differences to be lessened, especially with higher serum concentrations of DCLHb.[77] Jahr et al. found that the average difference between measured and calculated (actual) lactate levels could be an underestimation of between −2.2 mg/dL and −12 mg/dL when measured in the presence of an oxidizing hemoglobin agent outside the protective red blood cell. At clinically relevant concentrations of HBOC infusions (1-2 g/dL), the average difference was −2.2 mg/dL.

TABLE 6. US DCLHb Clinical Trial Lactate Data Based on Treatment Group and Survival Status (mg/dL)

Time Point	DCLHb	NS	*p*
Survival status: survivors			
LA 1	52.9 ± 35.1	58.4 ± 34.5	NS
LA 2	38.5 ± 21.5	29.4 ± 17.3	NS
LA 3	29.0 ± 22.2	27.5 ± 13.0	NS
LA 4	22.5 ± 18.1	25.5 ± 18.0	NS
LA 5	16.0 ± 8.0	19.5 ± 17.7	NS
Survival status: nonsurvivors			
LA 1	80.8 ± 39.2	87.0 ± 43.4	NS
LA 2	74.1 ± 42.8	73.0 ± 41.6	NS
LA 3	69.0 ± 23.6	66.4 ± 29.1	NS
LA 4	60.5 ± 22.8	130.2 ± 117.8	NS
LA 5	32.3 ± 9.4	39.6 ± 0.0	NS

Adjusting for this difference in the measured lactate levels of DCLHb-treated patients still yielded no significant difference in lactate levels between treatment groups at any time point.

It has been suggested that the BD and lactate response be analyzed with respect to volume of DCLHb infused.[7,9] A stratified analysis based on DCLHb volume is not possible, as the majority (86%) of patients received only two units of DCLHb during their resuscitation.

This work is consistent with the BP analysis from these two DCHLb clinical trials, which suggests the lack of an adverse pressor effect as seen by the absence of consistently or markedly elevated BP values with DCLHb resuscitation.[14,22] Several important facts were observed in the DCLHb BP publication. Although mean SBP and diastolic BP values differed at 2 of the 10 measured time points, BP curve analysis showed no SBP, diastolic BP, or mean arterial pressure differences based on treatment. Although SBP values ≥160 and ≥120 mm Hg were 2.2× and 2.6× more frequently noted in survivors, they were not more common

TABLE 7. US DCLHb Clinical Trial Lactate Distribution Data Based on Survival Status

	Lactate (mg/dL)				
Time Point	>90	61–90	31–60	10–30	<10
LA1 (p = 0.035)					
Died	8 (29.6)	11 (40.7)	7 (25.9)	1 (3.7)	0 (0.0)
Survived	10 (17.5)	10 (17.5)	25 (43.9)	10 (17.5)	2 (3.5)
LA2 (p = 0.001)					
Died	3 (23.1)	5 (38.5)	3 (23.1)	2 (15.4)	0 (0.0)
Survived	0 (0.0)	5 (9.4)	21 (39.6)	24 (45.3)	3 (5.7)
LA3 (p = 0.001)					
Died	2 (22.2)	3 (33.3)	3 (33.3)	1 (11.1)	0 (0.0)
Survived	0 (0.0)	3 (5.9)	15 (29.4)	27 (52.9)	6 (11.8)
LA4 (p = 0.001)					
Died	2 (33.3)	1 (16.7)	3 (50.0)	0 (0.0)	0 (0.0)
Survived	0 (0.0)	3 (5.9)	13 (25.5)	24 (47.1)	11 (21.6)
LA5 (p = 0.007)					
Died	0 (0.0)	0 (0.0)	3 (60.0)	2 (40.0)	0 (0.0)
Survived	0 (0.0)	2 (4.7)	3 (7.0)	24 (55.8)	14 (32.6)
	≥45	<45	OR	95% CI	
LA1					
Died	24 (88.9)	3 (11.1)	11.56	2.18–42.0	0.001
Survived	27 (47.4)	30 (52.6)			
LA2					
Died	11 (84.6)	2 (15.4)	13.79	2.91–128	0.001
Survived	13 (24.5)	40 (75.5)			
LA3					
Died	7 (77.8)	2 (22.2)	14.15	3.13–197	0.001
Survived	7 (13.7)	44 (86.3)			
LA4					
Died	5 (83.3)	1 (16.7)	13.36	3.18–1018	0.001
Survived	6 (11.8)	45 (88.2)			
LA5					
Died	0 (0.0)	5 (100)	0.13	0.00–25.7	NS
Survived	3 (7.0)	40 (93.0)			

The values given in the parentheses represent percentage.
OR, odds ratio; CI, confidence interval.

with DCLHb use, nor in DCLHb patients who expired, in US study nonsurvivors, or in any EU study patients. SBP values ≥160 and ≥120 mm Hg were 2.8× and 1.3× more frequently noted in DCLHb survivors when compared with NS survivors. Only 3% of the BP variation noted could be attributed to DCLHb use, and as expected, injury severity and baseline physiologic status were stronger predictors.[18]

It is noteworthy that the times at which these BD and lactate values were obtained varied substantially, reflecting the variable clinical use of these laboratory tests, even in clinical trials.[8,16] This time variability is not thought to have caused the study of potential adverse perfusion effects to be altered in a way that renders the conclusions of this post-hoc analysis to be erroneous.

It has been suggested that the real adverse consequences of the proposed pressor effects associated with HBOC use in trauma resuscitation are the possibility that elevated BP may cause clots to be disrupted, leading to accelerated hemorrhage and higher mortality.[20] Two findings are of note from these DCLHb clinical trials. First, markedly elevated BPs were neither uniquely observed in patients treated with DCLHb nor was mortality related specifically to elevated BPs.[18] Second, analyses of hemorrhage adverse events and severe adverse events at the time that these clinical trials were halted did not demonstrate a greater frequency of hemorrhagic complications in DCLHb-treated patients (unpublished data).

Further work will analyze the effect of DCLHb use in these clinical trials on the ability of the shock index (heart rate/SBP) to predict the need for continued resuscitation as well as its relationship with mortality. Preliminary data suggest that the performance of the shock index is not adversely affected by DCLHb use.[78] This shock index analysis will augment our understanding of how DCLHb and other HBOCs might improve patient outcomes due to being able to optimally fluid resuscitate trauma patients using readily available clinical parameters such as heart rate and SBP.

In summary, DCLHb, a tetrameric HBOC, was not associated with adverse perfusion as measured by BD and lactate data. This finding, when considered with the observation that there was not a measurable pressor effect or hemorrhage complications with DCLHb use, suggests that future study of HBOCs in the resuscitation of traumatic shock patients should not necessarily be prevented due to pressor effect concerns.

APPENDIX

United States (US) DCLHb Clinical Efficacy Trial

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European Union (EU) DCLHb HOST Clinical Efficacy Trial

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REFERENCES

1. Moore EE, Cheng AM, Moore HB, Masuno T, Johnson JL. Hemoglobin-based oxygen carriers in trauma care: scientific rationale for the US multicenter prehospital trial. *World J Surg.* 2006;30:1247–1257.
2. Winslow RM. Current status of oxygen carriers ('blood substitutes'): 2006. *Vox Sang.* 2006;91:102–110.
3. Sloan EP. The clinical trials of diaspirin cross-linked hemoglobin (DCLHb) in severe traumatic hemorrhagic shock: the tale of two continents. *Intensive Care Med.* 2003;29:347–349.
4. Schubert A, O'Hara JF Jr, Przybelski RJ, et al. Effect of diaspirin crosslinked hemoglobin (DCLHb HemAssist) during high blood loss surgery on selected indices of organ function. *Artif Cells Blood Substit Immobil Biotechnol.* 2002;30:259–283.
5. Philbin N, Rice J, Gurney J, et al. A hemoglobin-based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) versus hetastarch (HEX) in a moderate severity hemorrhagic shock swine model with delayed evacuation. *Resuscitation.* 2005;66:367–378.
6. Torres Filho IP, Spiess BD, Barbee RW, Ward KR, Oldenhof J, Pittman RN. Systemic responses to hemodilution after transfusion with stored blood and with a hemoglobin-based oxygen carrier. *Anesth Analg.* 2005;100:912–920.
7. Alayash AI. Oxygen therapeutics: can we tame haemoglobin? *Nat Rev Drug Discov.* 2004;3:152–159.
8. Bloomfield EL, Rady MY, Esfandiari S. A prospective trial of diaspirin cross-linked hemoglobin solution in patients after elective repair of abdominal aortic aneurysm. *Mil Med.* 2004;169:546–550.
9. Garrioch MA, McClure JH, Wildsmith JA. Haemodynamic effects of diaspirin crosslinked haemoglobin (DCLHb) given before abdominal aortic aneurysm surgery. *Br J Anaesth.* 1999;83:702–707.
10. Lamy ML, Daily EK, Brichant JF, et al. Randomized trial of diaspirin cross-linked hemoglobin solution as an alternative to blood transfusion after cardiac surgery. The DCLHb Cardiac Surgery Trial Collaborative Group. *Anesthesiology.* 2000;92:646–656.
11. Sakai H, Hara H, Yuasa M, et al. Molecular dimensions of Hb-based O(2) carriers determine constriction of resistance arteries and hypertension. *Am J Physiol Heart Circ Physiol.* 2000;279:H908–H915.
12. Saxena R, Wijnhoud AD, Carton H, et al. Controlled safety study of a hemoglobin-based oxygen carrier, DCLHb, in acute ischemic stroke. *Stroke.* 1999;30:993–996.
13. Przybelski RJ, Daily EK, Kisicki JC, Mattia-Goldberg C, Bounds MJ, Colburn WA. Phase I study of the safety and pharmacologic effects of diaspirin cross-linked hemoglobin solution. *Crit Care Med.* 1996;24:1993–2000.
14. Winslow RM. Cell-free oxygen carriers: scientific foundations, clinical development, and new directions. *Biochim Biophys Acta.* 2008;1784: 1382–1386.
15. Schultz SC, Grady B, Cole F, Hamilton I, Burhop K, Malcolm DS. A role for endothelin and nitric oxide in the pressor response to diaspirin cross-linked hemoglobin. *J Lab Clin Med.* 1993;122:301–308.
16. Sloan EP, Koenigsberg M, Gens D, et al. Diaspirin cross-linked hemoglobin (DCLHb) in the treatment of severe traumatic hemorrhagic shock: a randomized controlled efficacy trial. *JAMA.* 1999;282:1857–1864.
17. Kerner T, Ahlers O, Veit S, Riou B, Saunders M, Pison U. DCL-Hb for trauma patients with severe hemorrhagic shock: the European "On-Scene" multicenter study. *Intensive Care Med.* 2003;29:378–385.
18. Sloan EP, Philbin NB, Koenigsberg MD, Gao W. The lack of consistent DCLHb infusion blood pressure effects in the US and EU Traumatic Hemorrhagic Shock Clinical Trials. *Shock.* Published ahead-of-print May 18, 2009.
19. Abramson D, Scalea TM, Hitchcock R, Trooskin SZ, Henry SM, Greenspan J. Lactate clearance and survival following injury. *J Trauma.* 1993;35:584–588.
20. Cairns CB, Moore FA, Haenel JB, et al. Evidence for early supply independent mitochondrial dysfunction in patients developing multiple organ failure after trauma. *J Trauma.* 1997;42:532–536.
21. Claridge JA, Crabtree TD, Pelletier SJ, Butler K, Sawyer RG, Young JS. Persistent occult hypoperfusion is associated with a significant increase in infection rate and mortality in major trauma patients. *J Trauma.* 2000;48:8–14; discussion 14–15.
22. Krishna G, Sleigh JW, Rahman H. Physiological predictors of death in exsanguinating trauma patients undergoing conventional trauma surgery. *Aust N Z J Surg.* 1998;68:826–829.
23. Rixen D, Siegel JH. Bench-to-bedside review: oxygen debt and its metabolic correlates as quantifiers of the severity of hemorrhagic and post-traumatic shock. *Crit Care.* 2005;9:441–453.
24. Zehtabchi S, Baron BJ. Utility of base deficit for identifying major injury in elder trauma patients. *Acad Emerg Med.* 2007;14:829–831.
25. Reynolds PS, Barbee RW, Ward KR. Lactate profiles as a resuscitation assessment tool in a rat model of battlefield hemorrhage resuscitation. *Shock.* 2008;30:48–54.
26. Paladino L, Sinert R, Wallace D, Anderson T, Yadav K, Zehtabchi S. The utility of base deficit and arterial lactate in differentiating major from minor injury in trauma patients with normal vital signs. *Resuscitation.* 2008;77:363–368.
27. Gutierrez G, Comignani P, Huespe L, et al. Central venous to mixed venous blood oxygen and lactate gradients are associated with outcome in critically ill patients. *Intensive Care Med.* 2008;34:1662–1668.
28. Schmelzer TM, Perron AD, Thomason MH, Sing RF. A comparison of central venous and arterial base deficit as a predictor of survival in acute trauma. *Am J Emerg Med.* 2008;26:119–123.
29. Handrigan MT, Bentley TB, Oliver JD, Tabaku LS, Burge JR, Atkins JL. Choice of fluid influences outcome in prolonged hypotensive resuscitation after hemorrhage in awake rats. *Shock.* 2005;23:337–343.
30. Stern S, Rice J, Philbin N, et al. Resuscitation with the hemoglobin-based oxygen carrier, *HBOC*-201, in a swine model of severe uncontrolled hemorrhage and traumatic brain injury. *Shock.* 2009;31:64–79.
31. Gurney J, Philbin N, Rice J, et al. A hemoglobin based oxygen carrier, bovine polymerized hemoglobin (HBOC-201) versus Hetastarch (HEX) in an uncontrolled liver injury hemorrhagic shock swine model with delayed evacuation. *J Trauma.* 2004;57:726–738.
32. Pape A, Kleen M, Kemming G, Meisner F, Meier J, Habler O. Fluid resuscitation from severe hemorrhagic shock using diaspirin cross-linked hemoglobin fails to improve pancreatic and renal perfusion. *Acta Anaesthesiol Scand.* 2004;48:1328–1337.
33. Schultz SC, Hamilton IN Jr, Malcolm DS. Use of base deficit to compare resuscitation with lactated Ringer's solution, haemaccel, whole blood, and diaspirin cross-linked hemoglobin following hemorrhage in rats. *J Trauma.* 1993;35:619–625.
34. Winslow RM. Cell-free oxygen carriers: scientific foundations, clinical development, and new directions. *Biochim Biophys Acta.* 2008;1784: 1382–1386.
35. Natanson C, Kern SJ, Lurie P, Banks SM, Wolfe SM. Cell-free hemoglobin-based blood substitutes and risk of myocardial infarction and death: a meta-analysis. *JAMA.* 2008;299:2304–2312.
36. Spahn DR, Kocian R. Artificial O2 carriers: status in 2005. *Curr Pharm Des.* 2005;11:4099–4114.
37. Bone HG, Westphal M. The prospect of hemoglobin-based blood substitutes: still a long stony road to go. *Crit Care Med.* 2005;33:694–695.
38. Kim HW, Greenburg AG. Artificial oxygen carriers as red blood cell substitutes: a selected review and current status. *Artif Organs.* 2004;28: 813–828.

39. Greenburg AG, Kim HW. Hemoglobin-based oxygen carriers. *Crit Care.* 2004;8(suppl 2):S61–S64.
40. Chang TM. Hemoglobin-based red blood cell substitutes. *Artif Organs.* 2004;28:789–794.
41. Reah G, Bodenham AR, Mallick A, Daily EK, Przybelski RJ. Initial evaluation of diaspirin cross-linked hemoglobin (DCLHb) as a vasopressor in critically ill patients. *Crit Care Med.* 1997;25:1480–1488.
42. Rice J, Philbin N, Handrigan M, et al. Vasoactivity of bovine polymerized hemoglobin (HBOC-201) in swine with traumatic hemorrhagic shock with and without brain injury. *J Trauma.* 2006;61:1085–1099.
43. Smani Y, Faivre B, Audonnet-Blaise S, Labrude P, Vigneron C. Hemoglobin-based oxygen carrier distribution inside vascular wall and arterial pressure evolution: is there a relationship? *Eur Surg Res.* 2005;37:1–8.
44. Buehler PW, Alayash AI. All hemoglobin-based oxygen carriers are not created equally. *Biochim Biophys Acta.* 2008;1784:1378–1381.
45. Alayash AI, D'Agnillo F, Buehler PW. First-generation blood substitutes: what have we learned? Biochemical and physiological perspectives. *Expert Opin Biol Ther.* 2007;7:665–675.
46. Alayash AI. Hemoglobin-based blood substitutes: oxygen carriers, pressor agents, or oxidants? *Nat Biotechnol.* 1999;17:545–549.
47. Winslow RM. Alphaalpha-crosslinked hemoglobin: was failure predicted by preclinical testing? *Vox Sang.* 2000;79:1–20.
48. Sauaia A, Moore FA, Moore EE, Norris JM, Lezotte DC, Hamman RF. Multiple organ failure can be predicted as early as 12 hours after injury. *J Trauma.* 1998;45:291–301; discussion 301–303.
49. Cerovic O, Golubovic V, Spec-Marn A, Kremzar B, Vidmar G. Relationship between injury severity and lactate levels in severely injured patients. *Intensive Care Med.* 2003;29:1300–1305.
50. Eberhard LW, Morabito DJ, Matthay MA, et al. Initial severity of metabolic acidosis predicts the development of acute lung injury in severely traumatized patients. *Crit Care Med.* 2000;28:125–131.
51. Rixen D, Siegel JH. Metabolic correlates of oxygen debt predict posttrauma early acute respiratory distress syndrome and the related cytokine response. *J Trauma.* 2000;49:392–403.
52. Rixen D, Raum M, Bouillon B, Lefering R, Neugebauer E; Arbeitsgemeinschaft "Polytrauma" of the Deutsche Gesellschaft fur Unfallchirurgie. Base deficit development and its prognostic significance in posttrauma critical illness: an analysis by the trauma registry of the Deutsche Gesellschaft fur unfallchirurgie. *Shock.* 2001;15:83–89.
53. Cohn SM, Nathens AB, Moore FA, et al; StO_2 in Trauma Patients Trial Investigators. Tissue oxygen saturation predicts the development of organ dysfunction during traumatic shock resuscitation. *J Trauma.* 2007;62:44–54; discussion 54–55.
54. Baron JF. Blood substitutes. Haemoglobin therapeutics in clinical practice. *Crit Care.* 1999;3:R99–R102.
55. Carmichael FJ, Ali AC, Campbell JA, et al. A phase I study of oxidized raffinose cross-linked human hemoglobin. *Crit Care Med.* 2000;28:2283–2292.
56. Hill SE, Gottschalk LI, Grichnik K. Safety and preliminary efficacy of hemoglobin raffimer for patients undergoing coronary artery bypass surgery. *J Cardiothorac Vasc Anesth.* 2002;16:695–702.
57. Cheng DC, Mazer CD, Martineau R, et al. A phase II dose-response study of hemoglobin raffimer (Hemolink) in elective coronary artery bypass surgery. *J Thorac Cardiovasc Surg.* 2004;127:79–86.
58. Jahr JS, Moallempour M, Lim JC. HBOC-201, hemoglobin glutamer-250 (bovine), Hemopure (Biopure Corporation). *Expert Opin Biol Ther.* 2008;8:1425–1433.
59. Olofsson C, Nygards EB, Ponzer S, et al. A randomized, single-blind, increasing dose safety trial of an oxygen-carrying plasma expander (Hemospan) administered to orthopaedic surgery patients with spinal anaesthesia. *Transfus Med.* 2008;18:28–39.
60. Smani Y. Hemospan: a hemoglobin-based oxygen carrier for potential use as a blood substitute and for the potential treatment of critical limb ischemia. *Curr Opin Investig Drugs.* 2008;9:1009–1019.
61. Moore EE, Moore FA, Fabian TC, et al; PolyHeme Study Group. Human polymerized hemoglobin for the treatment of hemorrhagic shock when blood is unavailable: the USA multicenter trial. *J Am Coll Surg.* 2008;23:1–13.
62. D'Agnillo F, Alayash AI. Site-specific modifications and toxicity of blood substitutes. The case of diaspirin cross-linked hemoglobin. *Adv Drug Deliv Rev.* 2000;40:199–212.
63. Gundersen SI, Chen G, Palmer AF. Mathematical model of NO and O2 transport in an arteriole facilitated by hemoglobin based O_2 carriers. *Biophys Chem.* 2009;143:1–17.
64. Jia Y, Alayash AI. Effects of cross-linking and zero-link polymerization on oxygen transport and redox chemistry of bovine hemoglobin. *Biochim Biophys Acta.* 2009;1794:1234–1242.
65. Sperry JL, Ochoa JB, Gunn SR, et al; Inflammation the Host Response to Injury Investigators. An FFP:PRBC transfusion ratio >/=1:1.5 is associated with a lower risk of mortality after massive transfusion. *J Trauma.* 2008;65:986–993.
66. Sperry JL, Frankel HL, Vanek SL, et al. Early hyperglycemia predicts multiple organ failure and mortality but not infection. *J Trauma.* 2007; 63:487–493; discussion 493–494.
67. Dutton RP, Mackenzie CF, Scalea TM. Hypotensive resuscitation during active hemorrhage: impact on in-hospital mortality. *J Trauma.* 2002;52: 1141–1146.
68. Davis JW, Shackford SR, Mackersie RC, Hoyt DB. Base deficit as a guide to volume resuscitation. *J Trauma.* 1988;28:1464–1467.
69. Rutherford EJ, Morris JA Jr, Reed GW, Hall KS. Base deficit stratifies mortality and determines therapy. *J Trauma.* 1992;33:417–423.
70. Dunne JR, Tracy JK, Scalea TM, Napolitano LM. Lactate and base deficit in trauma: does alcohol or drug use impair their predictive accuracy? *J Trauma.* 2005;58:959–966.
71. Chang MC, Rutherford EJ, Morris JA Jr. Base deficit as a guide to injury severity and volume resuscitation. *J Tenn Med Assoc.* 1993;86:59–61.
72. Freilich D, Pearce LB, Pitman A, et al. HBOC-201 vasoactivity in a phase III clinical trial in orthopedic surgery subjects—extrapolation of potential risk for acute trauma trials. *J Trauma.* 2009;66:365–376.
73. Blow O, Magliore L, Claridge JA, Butler K, Young JS. The golden hour and the silver day: detection and correction of occult hypoperfusion within 24 hours improves outcome from major trauma. *J Trauma.* 1999;47:964–969.
74. Martin M, Murray J, Berne T, Demetriades D, Belzberg H. Diagnosis of acid-base derangements and mortality prediction in the trauma intensive care unit: the physiochemical approach. *J Trauma.* 2005;58:238–243.
75. Ivatury RR, Simon RJ, Havriliak D, Garcia C, Greenbarg J, Stahl WM. Gastric mucosal pH and oxygen delivery and oxygen consumption indices in the assessment of adequacy of resuscitation after trauma: a prospective, randomized study. *J Trauma.* 1995;39:128–134; discussion 134–136.
76. Manikis P, Jankowski S, Zhang H, Kahn RJ, Vincent JL. Correlation of serial blood lactate levels to organ failure and mortality after trauma. *Am J Emerg Med.* 1995;13:619–622.
77. Jahr JS, Osgood S, Rothenberg SJ, et al. Lactate measurement interference by hemoglobin-based oxygen carriers (Oxyglobin, Hemopure, and Hemolink). *Anesth Analg.* 2005;100:431–436.
78. Sloan EP, Weir B, Philbin N, Koenigsberg M. Shock index does not differ following the infusion of DCLHb in two clinical trials of traumatic hemorrhagic shock, such that its clinical use as a measure of compensated shock is valid. *Ann Emerg Med.* 2008;52:S109.