Artificial Organs

In Vitro Comparative Assessment of Mechanical Blood Damage Induced by Different Hemodialysis Treatments

*Ranko Sakota, *Carlo Alberto Lodi, *Sara Antonia Sconziano, †Werner Beck, and ‡Juan P. Bosch

*Research & Development, Gambro Dasco SpA, Medolla (MO), Italy; †Medical & Safety Office, Gambro Dialysatoren GmbH, Hechingen, Germany; and ‡Medical & Safety Office, Gambro, Washington, DC, USA

Abstract: Gradual deterioration of red blood cells (RBCs) due to mechanical stress (chronic hemolysis) is unavoidable during treatments that involve extracorporeal blood circulation, such as hemodialysis (HD). This effect is generally undetectable and does not generate any acute symptoms, but it leads to an increase in plasma free hemoglobin (*fHb*). There are no absolute safety levels for *fHb* increase, indicating the need for an empirical evaluation using comparative testing. The increase in *fHb* levels was investigated in vitro by applying double-needle double-pump HD (HD-DNDP), a new modality in which arterial and venous pumps both run continuously. *fHb* was measured during typical and worst-case simulated dialysis treatments (double-needle single-pump HD [HD-DNSP], hemodiafiltration [HDF-DN], single-needle double-pump HD [HD-SNDP], and HD-DNDP) performed in vitro using bovine blood for 4 h. Hemolysis-related indices (*fHb%*; index of hemolysis, *IH*; and normalized *IH*) were calculated and used for comparison. The increase in *fHb* during either HDF-DN or HD-SNDP with Artis and AK200 dialysis machines was similar, while the *fHb* at the maximum real blood flow rate (Qb_{real}) at the completion of the HD-DNDP treatment on Artis was higher than that for HD-DNSP using a Phoenix dialysis machine (*fHb%* = 1.24 ± 0.13 and 0.92 ± 0.12 for the Artis machine with HD-DNDP at Qb_{real} = 450 mL/min and Phoenix with HD-DNSP at Qb_{real} = 500 mL/min, respectively). However, the *fHb* levels increased linearly, and no steep changes were observed. The increases observed during HD-DNDP were the same order of magnitude as those for widely used bloodlines and treatment modes for delivering dialysis treatments. The observed results matched literature findings, and thus the measured *fHb* trends are not predicted to have clinical side effects. HD-DNDP treatment with Artis does not merit any additional concern regarding mechanical stress to RBCs compared with that observed for routinely used dialysis treatments, bloodlines and machines. Although the in vitro measurement of the *fHb* increase in bovine blood does not allow a prediction of the absolute level of blood mechanical damage or the possible effects in humans, such measurements are valuable for assessing hemolytic harm by performing tests comparing the proposed treatment with existing devices. **Key Words:** Hemolysis—Hemolysis index—Hemodialysis—Dialysis machine—Extracorporeal circuit—Blood pump—Plasma free hemoglobin—Patient safety.

Hemolysis is one of the most important and worrisome potential harms intrinsically related to extracorporeal treatments, particularly chronic hemodialysis (HD) (1). Hemolysis is usually defined as the release of hemoglobin into plasma caused by damage to red blood cell (RBC) membranes (2); this damage can be caused by chemical, thermal/radiation, or mechanical stress (3). It is important to clearly distinguish between acute and chronic hemolysis (1). Acute hemolysis, which develops immediately or within a few hours after exposure to a chemical, thermal, osmotic, or extreme mechanical hazard caused by manufacturing defect, machine failure, or operator error, is very unlikely to occur during dialysis treatment (1,4). In the case of massive RBC damage resulting in a significant decrease in the RBC count, noticeable signs and symptoms (e.g., back pain, headache, vomiting, agitation, blurring of vision, altered blood pressure, tachycardia, and shortness of breath) can be observed within hours in the patient (4).

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Address correspondence and reprint requests to Mr. Ranko Sakota, Research & Development, Gambro Dasco SpA, Via Modenese 66, I—41036 Medolla (MO), Italy. E-mail: ranko_sakota@baxter.com

R. Sakota and C.A. Lodi contributed equally to this work.

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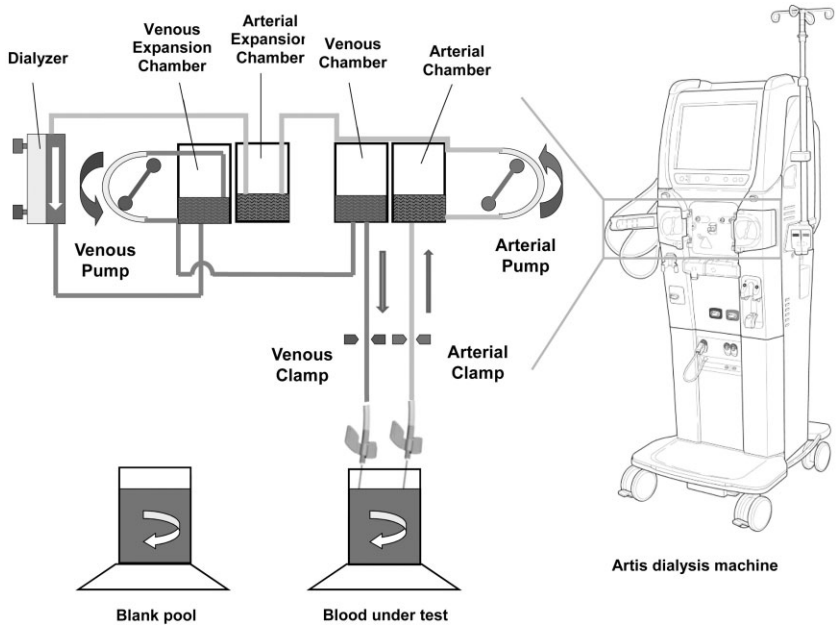

FIG. 1. In vitro test setup with the schematic of the extracorporeal circuit related to HD-DNDP treatment type (i.e., test ID ***c)*** in Tables 1 and 2). Blood pathways pre and post dialyzer are depicted in different shades of gray.

Chronic hemolysis is instead characterized by a gradual deterioration of RBCs that develops over time, for example, hours, weeks, or months, depending on the severity of the RBC damage, and is usually not detected during treatment. Mechanical stress to blood cannot be completely avoided during dialysis treatments because blood is circulated outside the body via one or two peristaltic pumps through an extracorporeal circuit that comprises several meters of bloodlines, including needles and chambers where the blood is in contact with air (1,3,5).

Gradual RBC deterioration due to mechanical stress (i.e., cell deformation [6] and/or cell membrane defects [7]) shortens RBC life spans. This so-called "sub-lethal hemolysis" is largely undetected because it falls below any acute hemolytic threshold (8,9) and because the patient's body is able to tolerate the resulting increase in plasma levels of free hemoglobin (*fHb*). No specific symptoms are present during or soon after treatment, with the exception of a possible worsening of chronic renal failure anemia over a period of weeks or months.

The aim of this investigation was to compare a new treatment, double-needle double-pump HD (HD-DNDP), to other treatment types widely used in clinics by measuring the increase in *fHb* during in vitro tests using bovine blood. In particular, the following treatment types were analyzed: HD and post-dilution hemodiafiltration using a double-needle single-pump (HD-DNSP and HDF-DN post, respectively), HD using a single-needle double-pump (HD-SNDP) and HD-DNDP. The latter is a specific treatment mode that was recently introduced for the Artis dialysis machine (Gambro Dasco SpA, Medolla, Italy); this treatment uses the same bloodline as the HD-SNDP treatment but with two pumps (arterial and venous) functioning simultaneously (see Fig. 1). The single-needle treatment is generally used in patients with critical vascular access (VA). However, in many cases, the treatment is started as double-needle (because it is commonly considered more efficient than single-needle) and then continued as single-needle if problems with VA occur (see [10,11]). With HD-DNDP, it is possible to start double-needle dialysis and, if needed, switch to single-needle HD (i.e., HD-SNDP) without changing the bloodline.

There are no literature reports of a configuration similar to HD-DNDP, with the exception of a prototype used on a very limited number of patients (12). Therefore, this investigation was performed as part of a safety and performance assessment regularly done prior to introduction of any treatment to the market.

MATERIALS AND METHODS

Blood preparation

All tests were performed using bovine blood under conditions simulating different types of HD treatments.

On each test day, blood from a single animal was obtained from a local slaughterhouse and immediately supplemented with ethylenediaminetetraacetic acid (EDTA, Carlo Erba, Rodano [MI], Italy, 2.5 g/L dissolved in saline) to prevent the formation of clots

or fibrin aggregates during transport. Subsequently, the blood was filtered through a stainless steel test sieve (Endecotts Ltd, London, UK, sieve aperture: 1 mm) and heparinized (5000 UI/L, Heparin Farmalabor Farmacisti Associati, Canosa di Puglia [BA], Italy).

Batches of up to 3 L of blood were used for each test session. Three liters of blood that was not subjected to dialysis treatment was kept as a blank reference to control for potential changes over time related to the intrinsic characteristics of the blood batches (e.g., spontaneous hemolysis). The test and reference pools were maintained in motion using a magnetic stirrer at 150 rpm and warmed to 37°C for the entire test duration.

Hematocrit (*Ht*) was measured after centrifugation in a microcentrifuge (Haematokrit by Hettich AG, Bäch, Switzerland) and varied from 35 to 40%, an appropriate range for the aim of these tests.

In vitro test setup

Dialysis machines equipped with an extracorporeal circuit specific to the treatment type were used (Fig. 1). Depending on the blood flow rate set value (Qb_{set}) and the treatment type, the extracorporeal circuit contained 15- or 16-G bore (i.e., inner diameter of 1.6 or 1.4 mm, respectively), 33-mm-long arterial and venous needles (15 L or 16 L Plume-S model, Hospal Industries, Meyzieu, France).

Comparative tests were performed for the HD-DNDP treatment (six runs). This treatment is only available for the Artis machine; thus, it was compared with the HD-DNSP treatment performed on a Phoenix machine (Gambro Dasco SpA, Medolla, Italy) because the blood pumping systems are equivalent in terms of the rotor and pump segments, allowing comparable maximum Qb_{real} to be obtained, and because the extracorporeal circuits are very similar (both have rigid blood chambers).

The same methodology was previously used before the introduction to the market of HDF-DN post and HD-SNDP treatments for the Artis machine. Those treatments were compared to the same ones on AK200 ULTRA S (Gambro Lundia AB, Lund, Sweden) dialysis machine, performing 6 and 24 runs, respectively.

The extracorporeal circuit, including a dialyzer (Polyflux series, Gambro Dialysatoren GmbH, Hechingen, Germany), was connected to the dialysis machine and primed with saline before dialysis began. To avoid coagulation and pressure problems related to ultrafiltration and ionic interactions with the dialysis fluid, the dialysate side of the dialysis filter remained filled but isolated from the flow during the HD treatments. Because it was not possible to isolate the dialysis filter during the HDF-DN post treatments, a re-infusion flow rate of 75 mL/min and a minimum net ultrafiltration rate (i.e., weight loss rate, 0.01 L/h) were applied. To replace lost weight, the blood pool was continuously refilled with an equivalent saline flow rate. The duration of dialysis was 4 h, which is the typical duration of a session. The setup data are summarized in Table 1 according to the different types of tests. Blood samples were drawn every 30 min from the extracorporeal circuit of the dialysis machine and from the blank pool using a sterile syringe. After the tests, blood disposal was performed according to European Regulation CE/1069/2009 (13).

***fHb* determination**

fHb was determined using the direct spectrophotometric absorption method (see, e.g., [6]). In this method, the percentage of plasma *fHb*, that is, *fHb%*, is determined by comparing the absorbance at 405 nm of plasma from centrifuged blood samples (Cambrex ELx808 Microplate Reader, BioTek Inc., Winooski, VT, USA) to a calibration curve of absorbance versus % hemolyzed blood. A calibration curve was constructed at the beginning of each test day using a linear regression of eight different dilutions (i.e., from 0 to 10%) of a completely hemolyzed plasma-containing blood sample: total hemolysis was performed by adding double-distilled water to whole blood, while plasma was obtained by centrifugation (Thermo Scientific centrifuge IEC CL30; 2000 rpm for 15 min) of a whole blood sample withdrawn before the test from the same blood. Hence, the *fHb%* at time *t* was calculated as follows:

$$fHb\%_t = \frac{y_t - q}{m} \quad (1)$$

where y_t is the absorbance reading at time *t* and *m* and *q* are the slope and intercept of the linear regression of the calibration curve, respectively.

In the literature, other types of formulas are used for in vitro testing to describe changes in *fHb* as indices of hemolysis (1,14). For the sake of completeness, the results will be presented using two commonly used indices: the index of hemolysis (*IH*) and the normalized index of hemolysis (*NIH*).

IH at time *t*, as defined in (15), is calculated from *fHb%* and *Ht*:

$$IH[\%]_t = (fHb\%_t - fHb\%_0) \cdot (1 - Ht_0) \cdot 100 \quad (2)$$

TABLE 1. *Hemolysis test parameters*

Parameter	In vitro test		
	a) Artis, HDF-DN post	b) Artis, HD-SNDP	c) Artis, HD-DNDP
Bloodline model	Artiset ULTRA HDF DN HC	Artiset HD SN HC	Artiset HD SN HC
Blood pool (L)	3.0	3.0	3.0
Blood flow rate (mL/min)	Qb_{set} = 350 Qb_{real} = 330	Qb_{set}*(mean)* = 210 Qb_{real}*(mean)* = 160	Qb_{set} = 315; 480 Qb_{real} = 300; 450
Treatment time (h)	4	4	4
Recirculations (with Qb_{real})	26.4	12.8	24.0; 36.0
No. of treatments	6	24	6
Needle (G)	16	16	16; 15
Dialyzer:	Polyflux 210H:	Polyflux 17L:	Polyflux 210H:
Surface area (m^2)	2.1	1.7	2.1
Fiber length (mm)	270	250	270
Fiber length (effective, mm)	297	297	297
Fiber inner diameter (μm)	215	215	215
Number of fibers	12 000	10 000	12 000
Quantities measured	*fHb* concentration and *Ht* variation	Same	Same
Comparison with	AK200 ULTRA S in HDF-DN post with BL 200 pre-post + UltraSteriSet, at the same Qb_{real} value	AK200 ULTRA S in HD-SNDP with BL 148 SN bloodline (6280341), at the same Qb_{real} *(mean)* value	Phoenix in HD-DNSP with Hospal cassette, Qb_{set} = 580 mL/min (Qb_{real} = 500 mL/min), 15 G needle
Aim of the test	Simulate a real HDF-DN dialysis and compare with a similar dialysis on AK200	Simulate a worst-case HD-SNDP dialysis (i.e., with $Qb_{set.arterial}$ = 380, $Qb_{set.venous}$ = 500 mL/min and stroke volume = 20 mL) and compare with a similar dialysis on AK200	Simulate a real and worst-case HD-DNDP dialyses and compare with worst-case dialysis on Phoenix

where $fHb\%_0$ and Ht_0 (expressed as a fraction of 1) refer to the initial condition at the beginning of the test.

NIH, proposed by Naito et al. (14), allows normalization of the data to the recirculation number; NIH_t is then the amount of *fHb* released per passage of blood volume through the device until time *t*:

$$NIH\,[g/100\ L]_t = (fHb\%_t - fHb\%_0)\cdot Hb_0\cdot(1-Ht_0)\cdot\frac{100}{RCN} = \Delta fHb_t\cdot(1-Ht_0)\cdot\frac{100\cdot V}{Qb_{real}\cdot\Delta t} \quad (3)$$

where Hb_0 is the initial hemoglobin concentration of whole blood (g/100 L), *RCN* is the number of blood volume recirculations, *V* is the blood pool volume (L), Δt is the test duration (min), and Qb_{real} is the real blood flow rate (L/min), which the dialysis machine estimates from Qb_{set} and the pre-blood pump pressure.

The values of the variations in *fHb* presented in the figures refer to the blood subjected to dialysis without adjustment for the variation in the blank pool.

As an additional marker, the *Ht* variation at the end of the treatment was assessed.

Statistical analysis

Data are presented as the mean ± standard deviation (SD). Data from different treatment types were compared using an independent two-tailed Student's *t*-test for equal or unequal variance based on the result of an F-test. Differences were considered significant at $P \le 0.05$. Data were analyzed using Minitab rel. 16.1.0 (Minitab Inc., State College, PA, USA).

RESULTS

Figures 2 and 3 show the mean values and SD of *fHb%* during the in vitro comparative dialysis for the HDF-DN post and HD-SNDP treatments, respectively. As expected, the *fHb%* values increased with time for all treatment types and machines.

Figure 4 shows the mean values and SD of *fHb%* during the in vitro comparative dialysis for the HD-DNDP treatment at two different Qb_{real} values on the Artis machine and for the HD-DNSP treatment at the maximum Qb_{real} on the Phoenix machine, that is, 500 mL/min.

The *fHb%* for Artis in HD-SNDP modality, using the same extracorporeal circuit as for HD-DNDP, at maximum Qb_{real} *(mean)* is close to the values observed in the HD-DNDP modality at a typical Qb_{real}.

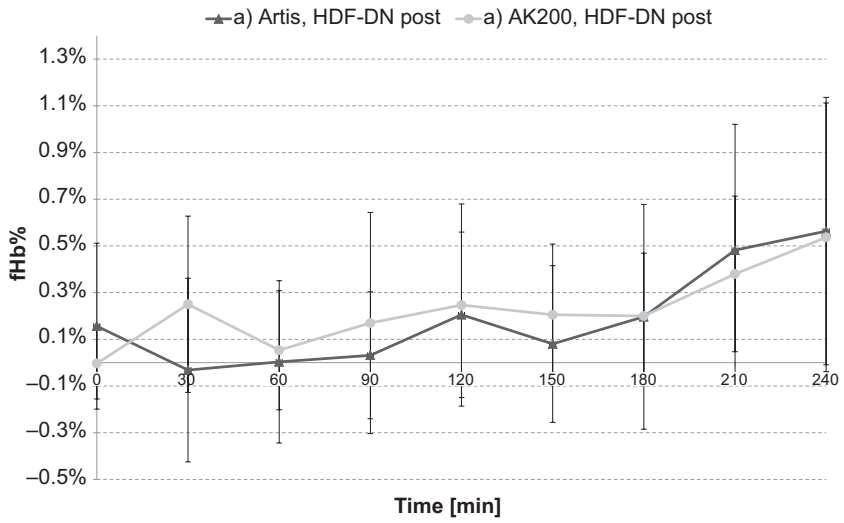

FIG. 2. Mean values and SD of plasma free hemoglobin [%] during HDF-DN post treatment on Artis and AK200 machines. Letters in the figure boxes refer to test ID listed in Tables 1 and 2. For the details of the test parameters, see Table 1.

The mean values and SD for the second analyzed hemolysis index, *IH*, follow the same *fHb%* trends because *IH* is only partly normalized with respect to *fHb%* (see Eq. 2); therefore, these values are not shown.

Data are summarized in Table 2 in numerical form. The values of Hb_0 and ΔfHb were indirectly calculated from other measured values: Hb_0 was derived from *Ht* and from a bovine mean corpuscular *Hb* concentration (MCHC) of 34 g/dL, the mean value of the data reported in previous studies (16) and (17), and ΔfHb was obtained by multiplying *fHb%* by Hb_0. In particular, the *Ht* variations in the blood pool over time during the in vitro tests were very limited and did not significantly differ with respect to the values of the corresponding blank pool. The differences in the Ht_0 values of the test and the blank are attributable to the variability of the reading and the partial dilution of the tested blood by residual priming solution in the bloodline. Moreover, Table 2 presents the results of the linearization of the *fHb%* mean values: in particular, the slope and correlation coefficient r^2 of the linear relationship used to fit the curves and the significant difference in the slope for each test on Artis versus AK200 or Phoenix. The linear fitting describes each curve well, as evidenced by r^2 values close to 1; the lowest r^2 values were obtained for the HDF-DN post treatments even when the slopes of the two curves were very similar based on the *P* value. A significant difference between slopes was observed only for HD-DNDP with maximum Qb_{real}, for which the slope was higher than that for HD-DNSP on Phoenix (slope = 0.006 vs. 0.004%/min, r^2 = 0.99 vs. 0.99, respectively).

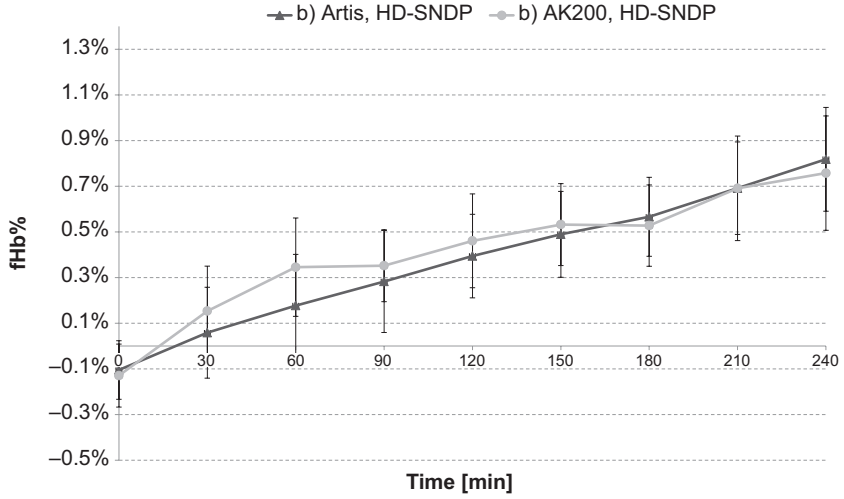

FIG. 3. Mean values and SD of plasma free hemoglobin (%) during HD-SNDP treatments on Artis and AK200 machines.

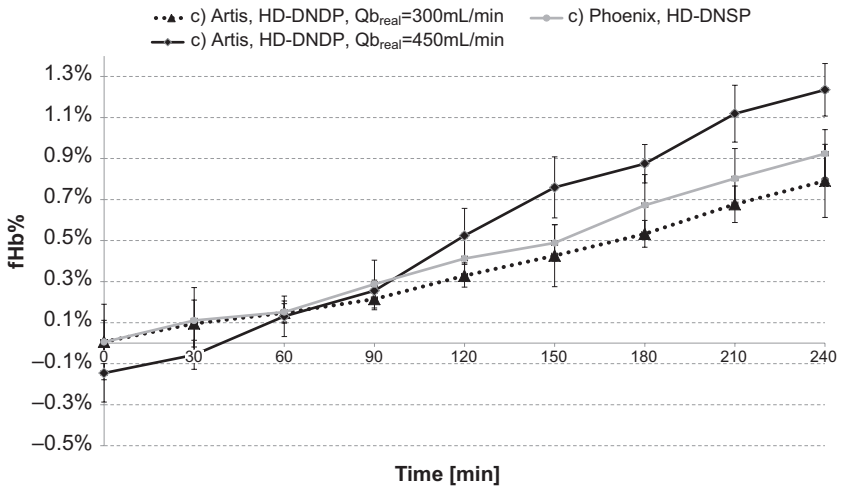

FIG. 4. Mean values and SD of plasma free hemoglobin (%) during HD-DNDP on Artis (Qb_{real} = 300 mL/min with 16 G needle, and Qb_{real} = 450 mL/min with 15 G needle) and HD-DNSP on Phoenix (Qb_{real} = 500 mL/min with 15 G needle).

DISCUSSION

The following conclusions can be drawn from the data summarized in Figs. 2–4 and Table 2.

The highest *fHb%* value was obtained during the HD-DNDP treatment at the maximum Qb_{real}, most likely due to the presence of two roller pumps running simultaneously in the arterial and venous parts of this blood circuit as well as the design complexity of the circuit itself, which contains 4 rigid chambers (arterial, venous, and two expansion chambers; see Fig. 1) and several rigid channels with curves. The lowest *fHb%* was observed for the HDF-DN post treatment, during which some *fHb* is most likely removed by convection. This hypothesis is supported by the dissociation of intact *Hb* molecules (tetramer with a molecular weight of 64 kDa) into smaller subunits (e.g., dimers) upon release from RBCs (18,19). The dimers could be removed by a high-flux membrane under convective treatment conditions.

The *fHb%* trends observed for Artis and AK200 are comparable for HDF-DN and HD-SNDP treatment types while that for the HD-DNDP treatment on Artis at maximum Qb_{real} is statistically different from the one for the HD-DNSP treatment on Phoenix at maximum Qb_{real}.

Thus, an extensive literature survey was performed to assess the relevance of the increase of mechanical blood damage observed for HD-DNDP treatment. Analysis of the literature is quite complex because the retrieved data were obtained both clinically and from experiments using either animal or human blood and because different indices were used to express hemolysis levels. In addition, many different limits have been proposed for the maximum *fHb*, depending on the duration of the specific device application (e.g., dialysis for chronic patients vs. patients with implanted cardiac devices). Consequently, although acute hemolysis is one of the most feared potential harms of extracorporeal blood treatment, there is no agreement on an absolute limit for an increase in *fHb* as an indicator of hemolysis, and no absolute safety levels have been identified (1,3,20,21). An empirical evaluation using comparative testing is therefore necessary (9,22,23).

Comparing our results (Table 2) to the data obtained in the literature survey reveals that the final *fHb%* in the presented in vitro tests appears to be within the limits specified by Yang and Lin (24) for humans (i.e., from 0.5 to 2.0%); the authors stated that a clinically acceptable *fHb%* should be less than 2% for a patient undergoing dialysis and less than 5% for a healthy person.

Furthermore, the *NIH* values obtained in our tests are lower than those measured by Kameneva et al. (9) using bovine blood, which are associated with a clinically accepted level of blood damage. In the experiments described by Kameneva et al., bovine blood was circulated by a roller pump at Qb = 300 or 450 mL/min for 2 h to compare three different VA devices: a subcutaneous VA (LifeSite), a dialysis catheter, and a 15 G fistula needle, yielding *NIH* values at Qb = 450 mL/min of 12.3 ± 1.8, 19.4 ± 2.8 and 14.6 ± 2.6, respectively (these *NIH* values were multiplied by 100 to obtain results consistent with Eq. 3). The authors concluded that the corresponding percentages of hemolysis were lower than those observed during tests using a subcutaneous VA device (Dialock) and a standard 16 G fistula needle (see (25)), for which an acceptable level of hemolysis exists. The final ΔfHb observed after the HD-DNDP treatment at maximum Qb_{real} (i.e., 1799 mg/L) would

TABLE 2. *Hemolysis tests results*

Test ID	Test setup	Under test		Blank		Under test						
		Ht_0 [%]	Ht_{final} [%]	Ht_0 [%]	Ht_{final} [%]	$fHb\%_{final}$	*fHb% linearization*		IH_{final} [%]	*NIH* [g/100 L]	Hb_0 [g/dL]	ΔfHb_{final} [mg/L]
							slope; r^2	*Diff.* vs. *ref.?*				
a)	Artis, HDF-DN post, Qb_{real} = 330mL/min, 4h	38	37	38	37	0.56 ± 0.57	0.002; 0.62	No ($P > 0.75$)	0.25 ± 0.50	1.23 ± 2.46	12.9	525 ± 1046
	AK200, HDF-DN post, Qb_{real} = 330 mL/min, 4 h	38	37			0.54 ± 0.58	0.002; 0.66	Reference	0.33 ± 0.30	1.64 ± 1.45	12.9	698 ± 618
b)	Artis, HD-SNDP, $Qb_{real(mean)}$ = 160 mL/min, 4 h	38	37	38	38	0.82 ± 0.23	0.004; 0.99	No ($P > 0.09$)	0.57 ± 0.14	5.78 ± 1.43	12.9	1192 ± 294
	AK200, HD-SNDP, $Qb_{real(mean)}$ = 160 mL/min, 4 h	38	37			0.76 ± 0.25	0.003; 0.91	Reference	0.55 ± 0.19	5.55 ± 1.92	12.9	1146 ± 397
c)	Artis, HD-DNDP, Qb_{real} = 300 mL/min, 4 h	36	35	37	37	0.79 ± 0.18	0.003; 0.98	No ($P > 0.06$)	0.50 ± 0.06	2.26 ± 0.42	12.2	961 ± 122
	Artis, HD-DNDP, Qb_{real} = 450 mL/min, 4 h	38	38	40	40	1.24 ± 0.13	0.006; 0.99	Yes ($P < 5{\cdot}10^{-4}$)	0.85 ± 0.11	3.11 ± 0.39	13.0	1799 ± 227
	Phoenix, HD-DNSP, Qb_{real} = 500 mL/min, 4 h	35	35	36	36	0.92 ± 0.12	0.004; 0.99	Reference	0.60 ± 0.13	1.77 ± 0.33	11.9	1086 ± 187

exceed the physiological *fHb* levels reported by Lund et al. (26) for bovine blood (i.e., 904 mg/L). However, when clinical data are considered, our values appear to be well below those measured during massive hemolysis events in dialysis patients (i.e., from 2900 to 30 000 mg/L, as reported in [5,27,28]). Moreover, the highest ΔfHb_{final} reported in Table 2 is the same order of magnitude as the value considered acceptable by Bernstein et al. (29) during in vivo experiments with dogs. The authors did not observe any effect for an *fHb* infusion of 0.1 mg/kg/min, but if the dose was doubled, *fHb* accumulation appeared. When these values are transposed to humans, an *fHb* infusion of 0.1 mg/kg/min would correspond to approximately 2057 mg/L of ΔfHb after 24 h, assuming that the blood volume is 7% of the body weight (30) (i.e., 0.1 mg/kg/min · 1440 min/(0.07 L/kg) = 2057 mg/L).

Comparing in vitro and in vivo results to predict the possible clinical effects of a new device or treatment mode is very difficult because the effects of blood damage differ significantly depending on the body reaction time, and a clear correlation between the extent of RBC damage (release of *fHb*) and clinical effects has not been established. First, caution is needed when drawing conclusions about clinical effects from data obtained using bovine blood because the mechanical fragility of human RBCs is greater than that of bovine RBCs (31).

Second, RBCs tend to relax after being exposed to mechanical stress (6,32). Due to the limited volume of the blood pool used in in vitro tests, the time between two consecutive blood passages through the extracorporeal circuit was less than the time for a dialysis treatment, that is, 7 min for HD-DNDP at maximum Qb_{real} versus approx. 23 min in vivo, potentially increasing the observed levels of *fHb* during the tests.

Third, in vivo, the body's compensatory mechanisms would limit the effects (i.e., *fHb* increase) observed during in vitro tests. In particular, damaged or morphologically altered RBCs are continuously re-absorbed by the spleen (30), and released *fHb* is bound as haptoglobin-hemoglobin complexes, which are cleared by hepatic mechanisms (14,29). According to Naito et al. (14), up to 1300 mg/L *fHb* may be bound in this manner in humans, and "if *fHb* exceeds this value, hemoglobinuria occurs and leads to organ failure."

In a situation producing massive blood damage, the hemolysis indices should increase by at least one order of magnitude more than the measured increase in the indices, given an identical or similar experimental setup. This effect should be obvious in the

graph as a steep change in the slope of the index curve. This opinion is confirmed by several studies (15,33–38) of hemolysis trends: no absolute threshold values have been identified, but it is commonly accepted that massive (acute) hemolysis occurs when the slope of the hemolysis index trend features an "acceleration," that is, it begins to increase significantly. By contrast, in our tests, the curve trends remained linear for all investigated extracorporeal circuits, treatments and dialysis machines (see Table 2).

The HD-DNDP treatment was developed to provide an adequate dialysis dose (39) to a patient with VA problems. The risk of a potential additional shortening of RBC life span in HD-DNDP would only be present when using the maximum Qb_{real}; therefore, this risk is limited because these critical patients are normally not treated with high Qb_{real} in clinical practice.

Based on this analysis, the HD-DNDP treatment on the Artis machine does not incur any additional concern regarding mechanical blood damage because the observed hemolytic indices varied over time in a linear manner and by the same order of magnitude as variations observed in vitro for other treatments on other devices that are already in clinical use. No safety issues in terms of hemolysis in vivo have been reported for these treatments and devices (e.g., the Phoenix dialysis machine, which has been in wide clinical use for more than 10 years). The in vitro measurement of the *fHb* increase described in the present study does not allow us to quantify the levels of hemolysis in patients because this phenomenon develops over time as a bodily reaction to RBC damage, while the increase in *fHb* is observed and measured immediately during laboratory tests. Nevertheless, *fHb* measurement is a valuable method to assess hemolytic harm by performing comparative tests of this treatment and existing devices using bovine blood.

Acknowledgments: This article is dedicated to the memory of Dr. Hans-Dietrich Polaschegg.

The work is based on a Gambro internal report for a meeting during which the presented hemolysis in vitro tests were assessed by Dr. H-D. Polaschegg. The meeting was held in Munich, Germany, on November 23, 2011. The internal report with the assessment conclusion was approved and signed by both Gambro and Dr. H-D. Polaschegg. Unfortunately, Dr. H-D. Polaschegg passed away on February 3, 2012; we are very grateful to him for his enthusiastic and substantial contribution to this work and other areas in which he was consulted; we therefore regret that we are unable to include him among the authors of this article.

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Conflict of Interest: R. Sakota, C.A. Lodi, and S.A. Sconziano have employment contracts with Gambro Dasco SpA. W. Beck and J.P. Bosch have employment contracts with Gambro AB. Gambro Dasco SpA and Gambro AB (including all direct and indirect subsidiaries of Gambro AB) are now a part of Baxter Healthcare Corporation.

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