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TRANSFUSION

ORIGINAL RESEARCH

Transfusion Practice

A validation study of the in vitro performance of hypoxic red blood cells for transfusion across centers in Europe

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[Correction added on 20 December 2025, after first online publication: The copyright line was changed.]

Abstract

Background: Refrigerated storage of red cell concentrate (RCC) leads to metabolic, oxidative, and structural changes that impair functionality and viability. These changes can be attenuated by hypoxic storage.

Objective: This study assessed the quality of leucocytes-reduced (LR), O_2/CO_2 reduced red blood cells (RBC) stored for 42 days after pre-storage O_2/CO_2 reduction with the Hemanext ONE System, to verify compliance with regional and blood center acceptance criteria across six sites.

Study design and methods: Validation studies of the in vitro performance of the LR, O_2/CO_2 reduced storage (Hemanext ONE) System were planned and executed at blood production centers in Italy, Germany, Norway and

Abbreviations: AS-3, additive solution formula 3; ATP, adenosine triphosphate; CPD, citrate phosphate double dextrose; DPG, diphosphoglycerate; FDA, Food and Drug Administration; GPX4, glutathione peroxidase 4; Hb, hemoglobin; HCT, hematocrit; HSB, Hemanext Storage Bag; LR, leucocytes-reduced; MDA, malondialdehyde; ORB, oxygen reduction bag; PAGGSM, phosphate-adenine-glucose-guanosine-saline-mannitol; RBC, red blood cell; RCC, red cell concentrate; ROS, reactive oxygen species; SC, sickle cell; SD, standard deviation.

Switzerland. The endpoint-associated study acceptance criteria included total hematocrit >50%, total hemoglobin ≥40 g and hemolysis <0.8%.

Results: In total, 133 whole blood units donated at six blood centers were evaluated after processing with the Hemanext ONE System and after 21 and 42 days of storage. At Day 1 post-processing, total hemoglobin (mean ± SD) was 52.4 g/unit ± 3.1 and total hematocrit percentage (mean ± SD) was 60.6% ± 7.0 (range, 51.9%–81.0%). Hemolysis at Day 42 of storage: overall mean ± SD, 0.26% ± 0.14.

Discussion: Hypoxic RBCs met regulatory quality criteria regardless of collection and processing modalities. These results indicate that processing of RBCs stored under hypoxic conditions satisfies acceptance criteria for transfusion into patients in six European regional blood centers.

KEYWORDS

hematology red cells, QA, RBC transfusion, regulatory

1 | INTRODUCTION

Once donated, whole blood is separated into a red cell concentrate (RCC) from which white blood cells, platelets, and plasma are typically removed. Isolated red blood cells (RBCs) are then resuspended in an acidic additive solution and stored under refrigerated conditions.[1] During storage of RCC in blood centers, gradual degradation of the RBCs occurs over time, referred to as the 'storage lesion'.[1] This degradation yields reduced quality RBCs with reduced function of the cells to offload oxygen in vitro,[2] as a result, RCC has a limited shelf life of 35–42 days.[2,3] Initial storage conditions such as temperature, additives and plasma removal, result in metabolic impairments of the RBC.[1] These impairments lead to the production or presence of nitric oxide scavengers,[4,5] free and non-transferrin bound iron,[5–7] and bioactive lipids.[5,8–10] In addition to metabolic impairments, oxidative damage due to prolonged exposure to oxygen can result in a decreased quality of RBCs characterized by increased stress mediators including thromboxane, leukotrienes and methemoglobin that contribute to decreased therapeutic potential.[1,11–14] Additionally, RBCs show progressively diminished deformability over time, a characteristic necessary to adequately perfuse the microcirculation.[1]

Previous methods to reduce oxidative stress during storage include experimental antioxidant-based additive solutions.[1,15–17] An emerging alternative to antioxidant-based solutions is hypoxic storage, whereby oxygen content of RCC units is reduced to low levels (less than 20% oxyhemoglobin [% SO_2]), prior to refrigeration and this oxygen saturation is then maintained throughout storage.[18] Hypoxic RBCs have been shown in vitro to attenuate the oxidative impairments that occur as blood is stored and improved post-transfusion recoveries in healthy autologous recipients.[2,19] Metabolomic analyses of hypoxic RBCs showed that levels of oxidative stress biomarkers, such as malondialdehyde (MDA),[20] are decreased and energy biomarkers including adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (DPG) are sustained for a longer time period in comparison to conventionally stored RBCs.[21] In pre-clinical studies, hypoxic RBCs stored for 3 weeks facilitate more effective resuscitation in animals sustaining hemorrhagic shock than 3-week-old conventionally stored RBCs.[22] Similar findings were also reported in a rat model of hemorrhagic shock plus traumatic brain injury.[23]

Hemanext ONE, a CE-certified and Food and Drug Administration (FDA)-authorized system,[24] was developed to process and store leucocytes-reduced (LR), O_2/CO_2 reduced RBCs under hypoxic conditions (Hemanext Inc., Lexington, MA). The primary goal of the Hemanext ONE System is to improve the safety and efficacy of transfusion therapy through a novel processing and storage method. This study aimed to evaluate the quality of hypoxically stored LR, O_2/CO_2 reduced RBCs over 42 days and ensure compliance with acceptance criteria at regional sites.

2 | MATERIALS AND METHODS

2.1 | Study design

In preparation for post-market investigations with patients requiring blood transfusions, a validation study

of the in vitro performance of the Hemanext ONE System was planned and executed at five regional blood centers: Genoa, Italy; Leipzig, Germany; Bergen and Oslo, Norway; and Bern and Lausanne, Switzerland. Bern and Lausanne are part of the same blood center and are therefore referred to as one center. This study was designed to allow the results to be applicable to RCC produced in regional blood centers. The study used units of LR-RBC in phosphate-adenine-glucose-guanosine-saline-mannitol (PAGGSM) additive solution prepared under conditions of same-day or overnight room temperature hold. The donation conformed to the inclusion criteria at each regional blood center and the units conformed to the Hemanext ONE System instructions for use at the time of inclusion in the study. All testing was conducted using calibrated laboratory equipment and instruments, according to site standard procedures or using a validated scientific method. A minimum of 90% of units tested should meet the required value to meet the validation acceptance criteria. All centers undertook one validation cycle, except the Swiss and German centers, where two and three validation cycles were performed, respectively. For the Swiss centers, one validation was performed in Lausanne (pilot site), the second validation cycle was a simple verification of the process with a reduced number of product and analyses, performed in the Production site in Bern. Both instruments and parameters for whole blood processing were the same across Swiss centers and Hemanext ONE procedure were identical at each site. The aim of the verification cycle is to ensure that the process can also be executed in the routine environment and that it leads to similar products.

2.2 | Donor and product inclusion criteria

Informed consent was obtained from all blood donors, and, before starting donation, the suitability of the donor was determined through medical history (according to local legislation). Donors had to meet the selection criteria complying with the respective national regulations. All sites tested for sickle cell (SC) trait as per the instructions for use.[24]

Whole blood units collected from donors were excluded from the study if: transfusion-transmitted diseases were detected; collection took ≥15 min to complete; collection was stopped prior to reaching the 450 ± 50 mL (Oslo, Bergen, Genoa, Lausanne and Bern) or 500 mL (Leipzig) acceptable volume range; SC trait was detected; or if there were grossly lipaemic specimens per visual inspection.

Additionally, whole blood units were considered non-evaluable if there was a protocol deviation during the study period, including storage deviation, incorrect mixing/agitation, product technical deviations such as cohesion of the oxygen reduction bag (ORB) or other deviations such as incorrect centrifugation.

2.3 | Blood samples

Each whole blood unit collected in the study generated one unit of O_2/CO_2-reduced LR-RBC. Whole blood processing, including leucocyte depletion, was performed within 20 h at ambient hold (20–24°C).[25] Whole blood was collected and processed using multiple modalities across centers: an automated blood processing system (Reveos; Terumo BCT Inc., Lakewood, CO), top-top and top-bottom methodologies (methodologies for each site are shown in Table 1). All units in this study were processed using the Hemanext ONE System and stored at 4°C immediately after transfer to the final storage bag, Hemanext Storage Bag (HSB), for 42 days (Figure 1). Validation testing commonalities across sites are shown in Table 2. The number of units assessed varied between centers, ranging from 17 units tested in Germany to 54 units tested in Norway, according to local requirements (shown in Table 1). In Switzerland, a total of 31 units were evaluated: 26 units in the Pilot Unit in Lausanne and 5 in the Production Unit in Bern. Blood bags were prepared with the same equipment and same procedure in both sites. Required analyses were performed in both Lausanne and Bern ($N = 31$) and additional analyses were performed only in Lausanne ($N = 26$).

2.4 | Study endpoint-associated acceptance criteria

The endpoint-associated study acceptance criteria at Day 1 after processing were total hematocrit (HCT) 50%–70% (>50% at Italian center only) and total Hb ≥40 g, and at Day 42 after processing hemolysis of <0.8% was required. Additionally, a negative result on bacterial testing at Day 42 was a requirement at German and Italian centers only. Additional key parameters measured without formal acceptance criteria were SO_2 in the Hemanext ONE System RCC unit after oxygen reduction: <20% with 80% confidence and 80% reliability after agitation at ambient temperature (20–26°C) for 3 h ± 15 min (recorded at German, Swiss, and Norwegian centers only), ATP (recorded at Italian, German, and Swiss centers only), MDA levels (recorded only at the Italian

TABLE 1 Methodology used in the collection and processing of whole blood by center.

Site	Methodology	Number of units	Free hemoglobin testing methods	Total HCT and total Hb testing methods
Bergen	Reveos top-top	33	HemoCue Plasma/Low Hb System (HemoCue AB Sweden)	Blood analyser Sysmex
Genoa	Top-bottom	30		
Lausanne/Bern	Top-top	31	Spectrophotometry NanoDrop™ 1000 (Thermo Fisher Scientific, Inc., Waltham, MA, USA) and Harboe method[50]	Blood analyser Sysmex
Leipzig	Top-bottom	17	Specord 50 Plus (Bioanalytics)	Specord 50 Plus (Bioanalytics)
Oslo	Top-bottom	20	HemoCue Plasma/Low Hb System (HemoCue AB Sweden)	Pentra XL 80 automatic haematology analyzer (Horiba Medical)

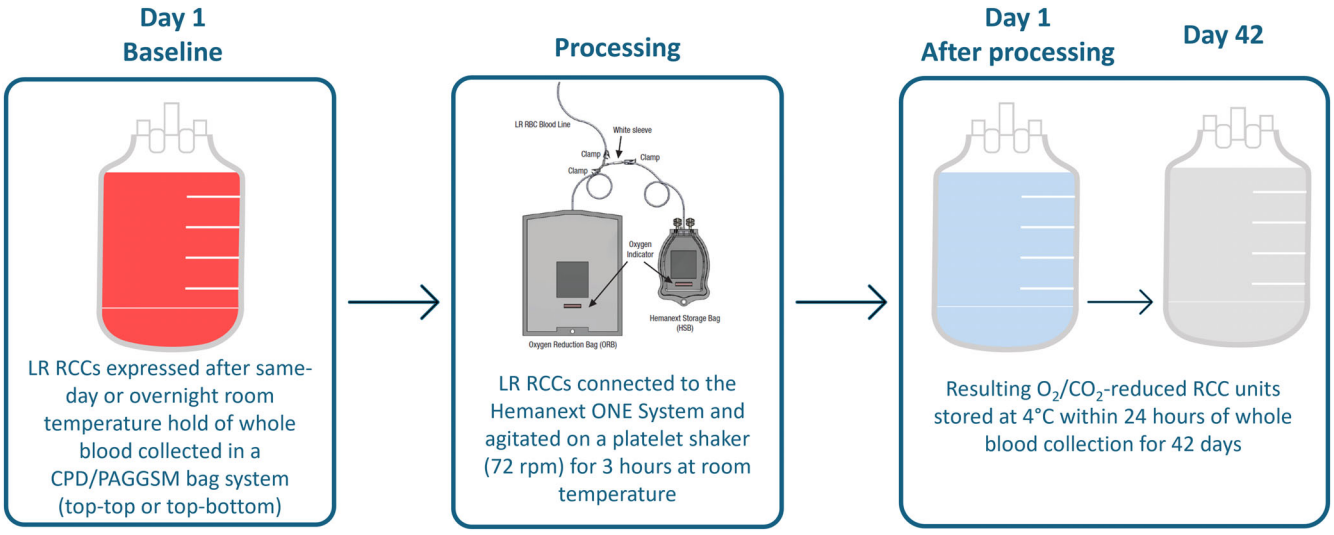

FIGURE 1 Blood processing schematic and parameters. Figure modified from Martin et al.[51] CPD, citrate phosphate double dextrose; Hb, hemoglobin; HCT, hematocrit; LR, leucoreduced; ORB, oxygen reduction bag; PAGGSM, phosphate-adenine-glucose-guanosine-saline-mannitol; RCC, red cell concentrate.

TABLE 2 Validation testing parameters: commonalities across centers.

	Italy	Norway[a]	Switzerland[b]	Germany
Total hematocrit (Day 1 after processing)	Yes[c] (>50%)	Yes[c] (50%–70%)	Yes[c] (50%–70%)	Yes[c] (50%–70%)
Hemolysis (Day 42)	Yes[c] (<0.8%)	Yes[c] (<0.8%)	Yes[c] (<0.8%)	Yes[c] (<0.8%)
Total hemoglobin (Day 1 after processing)	Yes[c] (≥40 g)	Yes[c] (≥40 g)	Yes[c] (≥40 g)	Yes[c] (≥40 g)
RCC unit volume	Yes[c]	Yes[c]	Yes[c]	Yes[c]
SO_2 (%)	No	Yes[d]	Yes	Yes
Residual leucocytes (cells/μL)	Yes[c] (≤1 × 10^6)	Yes (≤1 × 10^6)	Yes (≤1 × 10^6)	Yes (≤1 × 10^6)
Bacterial culture	Yes[c]	No	No	Yes[c]

[a]Including Bergen and Oslo centers.
[b]Including Bern and Lausanne centers.
[c]Acceptance criteria.
[d]Bergen center only.

center), lactate, glucose, and potassium concentrations (recorded only at the Swiss center), and pH (recorded only at the Swiss center). MDA measurement was carried out with TBARS (TCA Method) Colorimetric Assay Kit (Cayman Chemical, Michigan, USA) following the manufacturer instructions.[26]

3 | RESULTS

A total of 120 whole blood units donated at five blood centers were evaluated before processing with the Hemanext ONE System, after processing and after 21 and 42 days of storage. Methodology differences between centers are shown in Table 1. Key acceptance criteria (total HCT, hemolysis, and total Hb) for each site at Baseline, Day 21 and Day 42 are shown in Table 3 and Figure 2.

Total HCT percentage at Day 1 after processing (Figure 2A) was of 60.6% ± 7.0 across all sites, (range 51.9%–81.0%). The highest mean ± SD HCT was of 63.5% ± 3.4, recorded at the Lausanne center. Total Hb at Day 1 after processing (Figure 2C) at all centers was of 52.1 g/unit ± 5.3 (range 50.3–55.2 g/unit). The highest mean ± SD total Hb was 55.2 g/unit ± 5.0, recorded at the Italian center. Hemolysis at Day 1 after processing (Figure 2B) was of 0.3% ± 0.1 at all centers (range 0.1%–0.8%). At the Lausanne center, an initial increase of hemolysis was observed after the hypoxic processing (mean difference of 0.2%); additionally, one RCC had a hemolysis higher than 0.8% (0.8%) at the end of storage, this result remains within the validation acceptance

TABLE 3 Validation results from regional blood centers.

	Baseline[a]	After processing	Day 21	Day 42	Storage delta[b]
Hematocrit, mean (SD), %					
Leipzig	62.3 (2.5)	61.1 (2.4)	62.1 (2.8)[c]	63.1 (2.4)	
Genoa	62.1 (2.7)	61.4 (2.6)	66.3 (8.1)	63.4 (5.6)	
Bergen	58.7 (1.5)	57.3 (1.5)	–	57.2 (10.3)	
Oslo	57.6 (2.0)	57.6 (2.0)	–	55.5 (2.3)	
Lausanne/Bern[d]	60.0 (2.2)	59.5 (2.5)	–	63.5 (3.4)	
All centers	60.1 (2.8)	59.3 (2.8)	64.8 (6.9)	60.6 (7.0)	
Hemolysis, mean (SD), %					
Leipzig	0.11 (0.02)	0.13 (0.02)	0.19 (0.03)	0.25 (0.07)	0.12
Genoa	0.07 (0.03)	0.10 (0.03)	0.20 (0.09)	0.25 (0.09)	0.15
Bergen	0.12 (0.08)	0.06 (0.02)	–	0.17 (0.05)	0.11
Oslo	–	0.10 (0.03)	–	0.20 (0.04)	0.10
Lausanne/Bern[d]	0.10 (0.02)	0.28 (0.16)	–	0.41 (0.19)[e]	0.13
All centers	0.10 (0.05)	0.13 (0.11)	0.20 (0.07)	0.26 (0.14)	0.13
Hemoglobin, mean (SD), g/unit					
Leipzig	53.5 (4.7)	53.5 (4.8)	53.3 (4.8)	53.6 (4.5)	
Genoa	61.2 (4.8)	55.2 (5.0)	59.3 (7.6)	56.0 (4.9)	
Bergen	50.6 (5.0)	50.3 (5.0)	–	49.6 (5.0)	
Oslo	–	51.1 (5.6)	–	54.1 (2.1)	
Lausanne/Bern[d]	54.5 (4.9)	50.8 (4.8)	–	48.8 (4.6)	
All centers	55.1 (7.8)	52.1 (5.3)	56.3 (4.3)	52.4 (3.1)	

Note: Leipzig, $N = 17$; Genoa, $N = 30$; Bergen, $N = 33$; Oslo, $N = 21$; Lausanne, $N = 26$ ($N = 31$ at Day 42).

Abbreviations: LR, leucocytes-reduced; PAGGSM, phosphate-adenine-glucose-guanosine-saline-mannitol; RCC, red cell concentrate; SD, standard deviation.

[a]Baseline is defined as before processing with the Hemanext ONE System—LR-RCC (+PAGGSM).

[b]Storage delta = mean Day 42 value − mean Day 1 after processing value.

[c]Measurements were taken after 23 days of storage for logistical reasons, which is within current validation regulations (deviation of collection by ±3 days).

[d]Lausanne/Bern includes 26 units validated at the Lausanne center, Switzerland. A simplified verification of the hypoxic preparation process was performed with 5 more units at the Bern center, Switzerland. It included a reduced number of RCC units and analyses necessary to verify if the product complied to the specifications.

[e]One RCC had a hemolysis higher than 0.8% (0.81%) at the end of storage but this result remains within the validation acceptance criteria (a minimum of 90% of units tested should meet the required value).

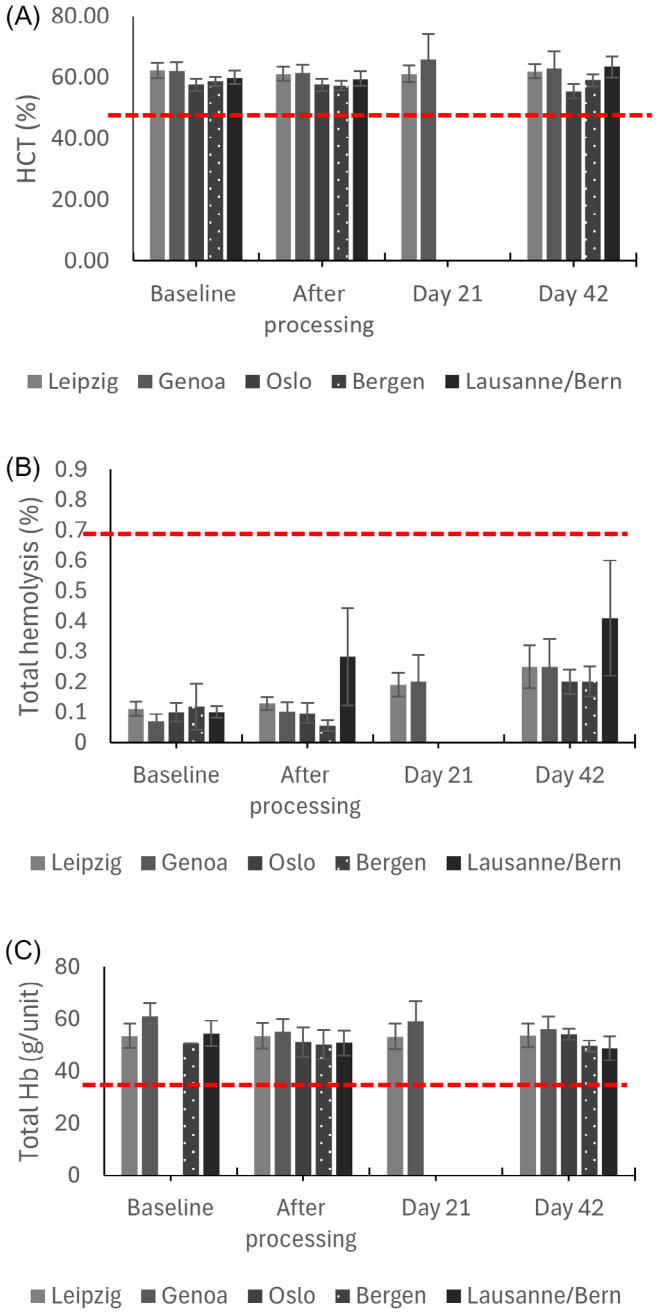

FIGURE 2 Values shown at Baseline, after processing, Day 21 and Day 42 at the five regional blood centers for: (A) total HCT content in function of processing and storage (mean ± SD total HCT [%]), (B) hemolysis function of processing and storage (mean ± SD percentage total hemolysis [%]), and (C) total hemoglobin content in function of processing and storage (mean ± SD total Hb [g/unit]). Leipzig, $N = 17$; Genoa, $N = 30$; Bergen, $N = 33$; Oslo, $N = 21$; Lausanne, $N = 31$. Day 21 data at the Leipzig center was reported at Day 23. Data were not reported at Day 21 at Oslo, Bergen, or Lausanne. Red dashed line indicates: (A) acceptance criteria of >50%, (B) acceptance criteria of <0.8%, (C) acceptance criteria of ≥40 g/unit. Hb, hemoglobin; HCT, hematocrit; SD, standard deviation.

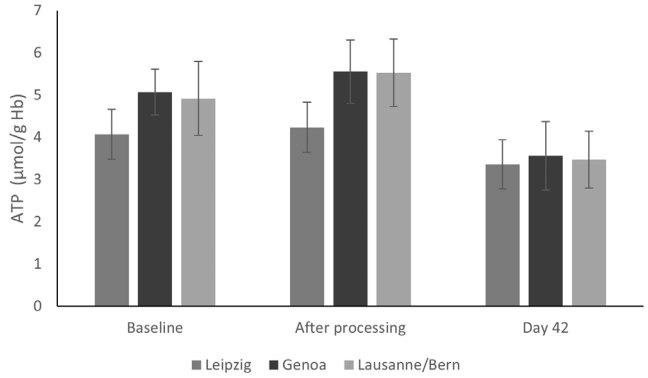

FIGURE 3 ATP levels in function of processing and storage. Mean ± SD ATP (µmol/g Hb) at Baseline, after processing with the Hemanext ONE System, at Day 42 at the Swiss, Italian, and German centers. Leipzig, $N = 17$; Genoa, $N = 30$; Bergen, $N = 33$; Oslo, $N = 21$; Lausanne, $N = 26$. ATP, adenosine triphosphate; Hb, hemoglobin; SD, standard deviation.

criteria (minimum of 90% of units tested should meet the required value). Total Hb at Day 42 of storage (Figure 2C) at all centers was of 52.4 g/unit ± 3.1 (range 40.4–61.9 g/unit). The highest mean ± SD total Hb was 56.0 g/unit ± 4.9, recorded at the Italian center. The lowest mean ± SD hemolysis was of 0.2% ± 0.1 recorded at the Bergen center. Additional acceptance criteria at Day 42 included bacterial culture (recorded at German and Italian centers). All recorded bacterial cultures were negative at Baseline and Day 42.

In addition to acceptance criteria, a number of other parameters were measured for information purposes. SO_2 in the Hemanext ONE System RCC unit after oxygen reduction were measured at German, Norway (Bergen), and Swiss centers. Potassium levels were measured at the Lausanne center (only performed in validation cycle number 1) where there was an increase at Day 42 compared with Baseline (53.00 ± 4.2 mmol/L vs. 2.37 ± 0.3 mmol/L, respectively); this was within ranges previously reported for RBCs which have been stored under hypoxic conditions.[2] In terms of extracellular potassium concentration, the RCCs under hypoxia studied in the present work are comparable to RCCs used in clinics,[27,28] and lower than values for g-irradiated products.[27] Mean ATP levels were recorded at Leipzig, Genoa and Lausanne centers at Baseline (before hypoxic processing), after hypoxic processing and at Day 42 (Figure 3). Mean ± SD ATP levels decreased between Baseline and Day 42 at all sites (Genoa: 5.07 ± 0.5 µmol/g Hb at Baseline vs. 3.56 ± 0.8 µmol/g Hb at Day 42; Lausanne: 4.92 ± 0.9 µmol/g Hb at Baseline vs. 3.47 ± 0.7 µmol/g Hb at Day 42; Leipzig: 4.07 ± 0.6 µmol/g Hb at Baseline vs. 3.36 ± 0.6 µmol/g Hb at Day 42). At the Italian center at Day 42, ATP levels were higher in hypoxic blood than in conventionally stored blood (Figure S1).

MDA was assessed at the Italian center before processing, at Baseline and at Day 42. MDA levels were lower in hypoxic blood than in conventionally stored blood, suggesting a protective effect of the Hemanext ONE System on RBC lipid peroxidation (Figure S2). Lactate and glucose levels were recorded at the Swiss center. Lactate levels (mean ± SD) increased between Day 1 post-processing and Day 42 (Lausanne: 4.2 ± 0.6 mmol/L at Baseline vs. 40.5 ± 2.8 mmol/L at Day 42). Glucose levels decreased or declined between Day 1 post-processing and Day 42 (Lausanne: 28.9 ± 0.7 mmol/L at Baseline and 7.3 ± 2.0 mmol/L at Day 42).

4 | DISCUSSION

This report documents the successful processing of hypoxic RBCs in multiple centers across Europe. Validations were performed in preparation for post-marketing clinical studies in thalassemia, sickle cell anemia, MDS, and burn patients. Despite slight variations in methodology between sites, all units measured passed the validation criteria; total HCT >50%, hemolysis of <0.8%, and total Hb ≥40 g/unit, at each site. At the Swiss centers, an initial increase of hemolysis was observed after the hypoxic processing and 3 h at ambient temperature (mean difference of 0.18%), the origin of which has not yet been identified. A further increase of hemolysis during storage was observed (mean difference between "Day 42" and "After processing" of 0.13%) and was similar to the differences reported in other centers (see Table 3). The mean hemolysis of the O_2/CO_2-reduced RCC at Day 42 reached 0.41% ± 0.19, which is higher than the data from the literature for conventional PAGGSM-stored RCC at Day 42 (0.13%[28] and 0.22%[27]), but well below the acceptance limit of 0.8%.

Variations in total Hb were observed between centers, with values ranging from 48.8 to 56.0 g/unit across centers at Day 42 after storage, and in HCT with values ranging from 55.5% to 63.5%. These variations between centers may be explained by differences in the workflow, for example, storage time between donation and centrifugation start, timing after end of separation, and filtration. Such variations are also routinely observed in conventional component preparation. This may also affect individual steps of the validation procedure during and after sampling. Additional factors which may contribute to total Hb variations are blood volume, centrifugation parameters (e.g., speed and time), and parameters of the press (timing, sensor position).

RCC storage in blood centers is a necessity for the logistics of blood transfusion. However, storage lesions of RBCs resulting from storage of up to 42 days have a detrimental effect on the function of the stored cells.[1,29] The observed changes in lactate and glucose levels during refrigerated storage are consistent with values found in previous studies in both hypoxic and conventional storage conditions.[19] These changes are due to ongoing cellular metabolism which is needed to maintain the important physiologic functions of the cells. Hemanext's research with several commercially available additive solutions demonstrated that Additive Solution Formula 3 (AS-3) and PAGGSM were the optimal choices for hypoxically stored RBCs.[30] Hypoxic RBCs processed with these additive solutions demonstrated the highest levels of adenosine diphosphate, ATP, and 2,3-diphosphoglycerate, the key metabolites for maintaining the functionality and survival of the RBCs, as well as acceptable levels of hemolysis, HCT, red cell recovery, and total Hb content per unit of RBC processed. As AS-3 is not available in the Europe, PAGGSM was utilized in the validation.

Oxidative stress is a major contributor to the series of biochemical and morphological changes that occur to RBCs during storage, leading to a decrease in levels of energy biomarkers such as ATP, and an increase in oxidation biomarkers such as MDA, a lipid peroxidation product.[20,23,29,31] ATP is important for the energy-dependent maintenance of structural integrity of the RBCs during periods of circulatory stress.[32] Consequently, a decrease in ATP is associated with alterations in RBCs that lead to loss of deformability, reducing the ability of RBCs to navigate micro-vessels and deliver O_2 efficiently.[32–34] ATP levels in RBC transfusion are critical for the survival and functionality of stored RBCs; high ATP levels in stored blood improve post-transfusion outcomes by reducing hemolysis and enhancing O_2 delivery.[35–37] MDA is one of the main products of polyunsaturated fatty acids peroxidation and has been widely used as marker of RBC membrane lipid peroxidation.[38–40] Previous studies have shown that MDA levels tend to increase over time in standard storage,[29] which is associated with a reduction in RBC deformability.[20,38,39] Indeed, increased RBC MDA levels enhance erythrophagocytosis, resulting in rapid removal of RBCs with high MDA levels from the peripheral circulation.[41] Thus, optimizing ATP and preventing the storage-induced increase in RBC MDA levels might result in the improvement of the quality of stored RBCs and transfusion yield with a positive impact on patient outcomes.[42]

In the performed validations, ATP and MDA levels were measured at one center as key energy and lipid peroxidation/oxidative stress biomarkers and were found to be increased and decreased respectively by hypoxic storage. During conventional RBC storage, ATP levels initially rise before falling below pre-storage levels; the level

of this decrease is dependent on a number of factors, such as the composition of the storage solution and pH of the RCC suspension.[33,43] Conversely, hypoxic blood has been shown to increase ATP up to 42 days of storage compared to conventional RBCs.[44] The ATP concentration at the end of storage is weakly correlated with the in vivo recovery of the RBCs.[44] Hypoxic conditions stimulate glycolysis, the pathway producing ATP in mature RBCs.[2,19] This supports that ATP-dependent processes remain functional even in low oxygen environments. The maintenance of ATP levels in hypoxic conditions could enhance RBC recovery and circulation efficiency during transfusion.[2,21,45]

Lower MDA levels at Day 42 versus conventionally stored RBCs show hypoxic storage is protective against RBC membrane lipid peroxidation. As MDA levels tend to increase during standard storage, strategies to maintain low RBC MDA levels during RBC storage for blood banking purposes are crucial to improve RBC transfusion yield.[2,20]

Lipid peroxidation during RCC storage has been linked to cell-free Hb in plasma, a marker of membrane damage.[20,42] Ferroptosis is a peroxidation-driven regulated form of cell death that requires accessible cellular iron, that has been implicated in RBC storage and longevity.[46] The process is driven by loss of activity of the lipid repair enzyme glutathione peroxidase 4 (GPX4), leading to an accumulation of lipid-based reactive oxygen species (ROS), particularly lipid hydroperoxides.[47] Additionally, the expression of Steap3, a ferrireductase, has been shown to affect oxidative damage during the storage of RBCs in a murine study.[48] Increased levels of Steap3 resulted in degradation of cellular membrane through lipid peroxidation, leading to failure of RBC homeostasis and hemolysis. Ferroptosis-like mechanisms may contribute to hemolysis during RBC storage, particularly under oxidative stress conditions.[46–49] By minimizing ferroptosis-related damage, through the reduction of oxygen availability and thus limiting ROS and lipid peroxidation, hypoxic storage conditions may improve RBC membrane stability and reduce hemolysis. Thus, maintaining low MDA RBC levels with the Hemanext ONE System may preserve RBC features with a possible beneficial effect on RBC transfusion yield, in comparison to established blood banking procedures. This is crucial in chronically transfused patients such as thalassaemic or myelodysplastic patients.

In summary, hypoxic RBCs processed with the Hemanext ONE System met acceptance criteria for transfusion. Compared with conventionally stored blood, hypoxic RBCs maintained high levels of ATP (Italian center only), and attenuated MDA accumulation (Italian center only), an indirect estimate of RBC membrane oxidation.

5 | CONCLUSIONS

Hypoxic RBCs were successfully validated irrespective of the different modalities used to collect and process the RBCs. These results indicate that processing of RBCs stored under hypoxic conditions satisfies acceptance criteria for transfusion into patients at blood centers in four European countries. Additionally, supplemental data from the Italian site illustrated that hypoxic RBCs attenuate MDA accumulation as a marker of RBC lipid peroxidation. Moreover, the oxygen reduction process was easily performed in the routine environment. Based on prior research, and supported by data from this study, it is expected that deoxygenation of RBCs with the Hemanext ONE System will maintain more physiological levels of key blood quality parameters compared with conventionally stored RBCs. Clinical data are required to evaluate the benefit of O_2/CO_2-reduced RBCs over conventionally stored RBCs.

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CONFLICT OF INTEREST STATEMENT

VA has received speaker honoraria from CSL Behring, Werfen, Vifor, Bristol Myers Squibb and was a Hemanext-sponsored speaker at ISBT Gothenburg; GU, RH, THFL, EK, ED, AM and LDF have no conflicts of interest to declare; GH was a Hemanext-sponsored speaker at ISBT in Gothenburg, June 2023; MP has received research funding from Hemanext Inc. for R&D projects; GG was an independent consultant to Hemanext Inc. at the time of the study; IW and JW were employees of Hemanext Inc. at the time of the study; LO is an employee of Hemanext Inc.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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