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ORIGINAL PAPER

Effect of warming and flow rate conditions of blood warmers on red blood cell integrity

T. G. Poder,[1,2] D. Pruneau,[3] J. Dorval,[3] L. Thibault,[4] J.-F. Fisette,[1] S. K. Bédard,[1] A. Jacques[4] & P. Beauregard[3,5]

[1] *UETMIS, CIUSSS de l'Estrie – CHUS, Sherbrooke, QC, Canada*
[2] *CRCHUS, Sherbrooke, QC, Canada*
[3] *Blood bank, CIUSSS de l'Estrie – CHUS, Sherbrooke, QC, Canada*
[4] *Research and Development, Héma-Québec, Québec, QC, Canada*
[5] *Hematology-Oncology division, CIUSSS de l'Estrie – CHUS, Sherbrooke, QC, Canada*

Vox Sanguinis

Background and Objectives Fluid warmers are routinely used to reduce the risk of hypothermia and cardiac complications associated with the infusion of cold blood products. However, warming blood products could generate haemolysis. This study was undertaken to compare the impact of temperature of blood warmers on the per cent haemolysis of packed red blood cells (RBCs) heated at different flow rates as well as non-flow conditions.

Materials and Methods Infusion warmers used were calibrated at 41·5°C ± 0·5°C and 37·5°C ± 0·5°C. Cold RBC units stored at 4°C in AS-3 (n = 30), aged 30–39 days old, were divided into half units before being allocated under two different scenarios (i.e. infusion pump or syringe).

Results Blood warmers were effective to warm cold RBCs to 37·5°C or 41·5°C when used in conjunction with an infusion pump at flow rate up to 600 ml/h. However, when the warmed blood was held in a syringe for various periods of time, such as may occur in neonatal transfusions, the final temperature was below the expected requirements with measurement as low as 33·1°C. Increasing the flow with an infusion pump increased haemolysis in RBCs from 0·2% to up to 2·1% at a flow rate of 600 ml/h regardless of the warming device used (P < 0·05). No relevant increase of haemolysis was observed using a syringe.

Conclusions The use of a blood warmer adjusted to 41·5°C is probably the best choice for reducing the risk of hypothermia for the patient without generating haemolysis. However, we should be cautious with the use of an infusion pump for RBC transfusion, particularly at high flow rates.

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Introduction

In North America, red blood cell (RBC) units are stored at temperatures between 2°C and 6°C for up to 42 days [1, 2]. Transfusion of these blood products without being warmed might represent a risk of hypothermia for transfused patients, which can reduce patients' metabolism and might lead to arrhythmia or cardiac arrests [3, 4]. To prevent these adverse transfusion situations, RBC units are frequently warmed using fluid warmers. There is however a potential risk of RBC haemolysis associated with the use of these instruments [3, 5]. Very high level of haemolysis can lead to fever, kidney failure, disseminated intravascular coagulation, hypotension and coagulopathy [6–8]. In severe adverse reactions, a vasomotor shock and even patient death may also occur [6]. Regulatory agencies require that haemolysis per cent of blood products must not exceed 0·8% and 1% in Canada and Europe, and in the United States, respectively [1, 9, 10].

Indications for warming blood products (e.g. patient rewarming, massive transfusion, trauma situations,

Correspondence: Thomas G. Poder, UETMIS, Centre intégré universitaire de santé et de services sociaux de l'Estrie – Centre hospitalier universitaire de Sherbrooke (CIUSSS de l'Estrie – CHUS), Hôtel-Dieu de Sherbrooke J1G2E8, QC, Canada
E-mail: tpoder.chus@ssss.gouv.qc.ca
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transfusion for 30 min or more) are currently defined in the 'Guidelines for the use of Blood Warming Devices' of the American Association of Blood Banks [11]. Interestingly, no indications are included in these guidelines on what is the optimal temperature for warming RBC units although it is specified that haemolysis and damages to RBCs must be prevented by limiting heating to a temperature of 37°C. It is also well recognized that hypothermia following rapid transfusion of large volumes of refrigerated blood products can cause serious complications in patients [11, 12]. Studies on which these guidelines are based are, however, mostly focused on outdated approaches (i.e. water baths and other experimental procedures) that do not include modern commercial fluid warmers [5]. In addition, to our knowledge, the use of modern fluid warmers in combination with infusion pumps or syringes, commonly used in a hospital setting, has not been studied.

In our research, we have compared the use of two fluid warmers with countercurrent heat exchange set at two different temperatures: 37·5°C and 41·5°C. The main objective was to assess induced RBC haemolysis at these two temperatures. The consistency between the theoretical temperature of warming and the outlet temperature of the RBC units warmed was also investigated. This was done to assess a possible risk of hypothermia for the patient (i.e. at temperature below 35°C). All haemolysis and temperature tests were conducted under different flow rates and transfusion techniques to identify whether factors other than temperature were likely to influence the level of haemolysis in RBC units.

Materials and methods

Blood products

The RBC units used in this study were obtained from Héma-Québec. Prior to collection, blood donors had signed an informed consent form. Whole blood (450 mL) was collected in 63 mL of citrate phosphate double dextrose (CP2D) using Leukotrap WB system collection sets (Haemonetics, Braintree, MA, USA) and stored at 1–6°C. Within 72 h from collection, whole blood bags were first leucoreduced by filtration and then centrifuged at 5147 g for 10 min. After plasma expression, 100 mL of additive solution AS-3 was added to the RBCs and units were stored between 1 and 6°C. For the warming study, the age of RBC units ranged from 30 to 39 days of storage and their average volume was 334 ± 18 mL. The RBC units were all AB positive. As regards to haematocrit, total haemoglobin and free haemoglobin, RBC units ranged from 0·473 to 0·598 l/l, 156 to 201 g/l and 0·05 to 0·21 g/dl, respectively.

Study 1 – Fluid warmers in association with an infusion pump

The study was conducted using a prospective comparative design with matching to study the impacts of the warmers at different flow rates. 15 RBC units were used, and each unit was split into two identical units in a satellite polyvinyl chloride (PVC) bag. The fluid warmers (Hotline; Smiths Medical, Rockland, MA, USA) were set at 37·5°C and 41·5°C (models HL-90-38 and HL-90, respectively). These warmers have an identical mechanism and use hot water to warm a tube through which a product flows over a length of 20 cms (i.e. countercurrent heat exchange). The only difference is the warming temperature used (i.e. 37·5 vs. 41·5°C). All RBC half units were pumped through the fluid warmer using the infusion pump Symbiq (Hospira, Lake Forest, IL, USA). This pump uses a piston-actuated diaphragm cassette. Each RBC half unit was sequentially tested at a flow rate of 60 and 150 ml/h, followed by a 15-min stagnation period and resumed at flow rates of 150 ml/h and 600 ml/h. This corresponded to a single pass of the RBC unit through the fluid warmer during which the flow rate was adjusted. These flow rates match the current practices in our hospital and are similar to those used in other hospitals [13]. Specifically, a short survey conducted in our hospital over 1 month indicated that for adults the minimum flow rate using an infusion pump was 60 ml/h and the maximum was usually 450 ml/h, exceptionally 600 ml/h or higher. In the majority of cases, the flow rate used was 150–200 ml/h. In our hospital, we mainly use infusion pump for prophylactic transfusion to treat haematological anaemia, and sometimes for massive infusion when a compression sleeve is not available. In this study, at the inlet of the infusion tubing (volume of 66 ml), a macrofilter of 210 microns was used. There was no filter at the outlet of the infusion tubing. Samples of 13 ml were collected to measure haemolysis per cent. Each sample was first collected in the RBC unit and then at the outlet at the end of each applied flow rate. Outlet blood flow temperatures were measured for each step using a Fluke 52 Series II thermometer (Fluke Corporation, Everett, WA, USA). The temperature was measured continuously over a few seconds of flow and the final measure taken when the temperature was stable.

Study 2 – Mimicking neonate exchange transfusion

In this second study, the impact of the temperature setting of fluid warmer on RBC haemolysis was studied in a neonate transfusion exchange process. Again, 15 RBC split units were warmed through the hotline devices

following a comparative design. No infusion pump was used for this experiment, and the ambient temperature in the room was 23°C. At the inlet of the infusion tubing (volume of 46 mL), a macrofilter of 170–260 microns was used. There was no filter at the outlet of the infusion tubing and a 20-mL syringe aspirated warmed RBCs. A first thrust of 4 ml was performed at time 0 followed by two others after 2 and 5 min. The haemolysis per cent and the outlet temperatures were assessed as described for study 1. Samples to calculate haemolysis per cent were collected before warming and at the end of the experiment with a syringe. Temperature measurements were taken at the outlet of the warmer before use of a syringe and at each step of the experiment with a syringe. Figure 1 describes the design for both studies.

Haemolysis measurements

Haematocrit (Hct) and total haemoglobin were measured with on a Coulter AcT 5diff AL (Beckman Coulter, Miami, FL, USA). To measure free haemoglobin, samples were centrifuged at 5 000 g for 10 min and the supernatant was centrifuged a second time at 5 000 g for 7 min. The supernatant was next filtered through a 0·22-μM filter (Millipore, Billerica, MA, USA) to remove the remaining cellular debris and particulate matter (e.g. lipids) which may interfere with the optical measurement of free haemoglobin (FHb) level. FHb level was measured using a HemoCue Plasma/Low HB photometer (HemoCue, Angelholm, Sweden). The percentage of haemolysis was calculated with the following equation: ([FHb]/[Total Hb]) × (100 − Hct).

Statistical analysis

A statistical power test at 80% for a 95% significance indicated a minimum of 13 observations per group to detect a statistically significant difference between an initial level of 0·25% haemolysis and a final level of 0·8%, and 15 observations per group for a difference between final results of 0·8% and 1·2%. Concerning data analysis, a distribution normality test was performed for each variable (Shapiro–Wilk test). To compare the values of haemolysis obtained at different temperatures, the data were analysed with the Wilcoxon signed-rank test. To analyse the effect of control variables on test results, we performed least ordinary square (OLS) estimates using the data from the two studies. The data were compiled on MS Excel charts to be later transferred to the R statistical software (difference tests and box-plot) and STATA 14 (OLS estimates) softwares (R-Project; Austria and StataCorp, Texas, respectively). Any result whose significance was greater than 95% was considered significant.

Ethical considerations

The study was approved by the Ethics Research Committee of the University Hospital of Sherbrooke (CHUS). All

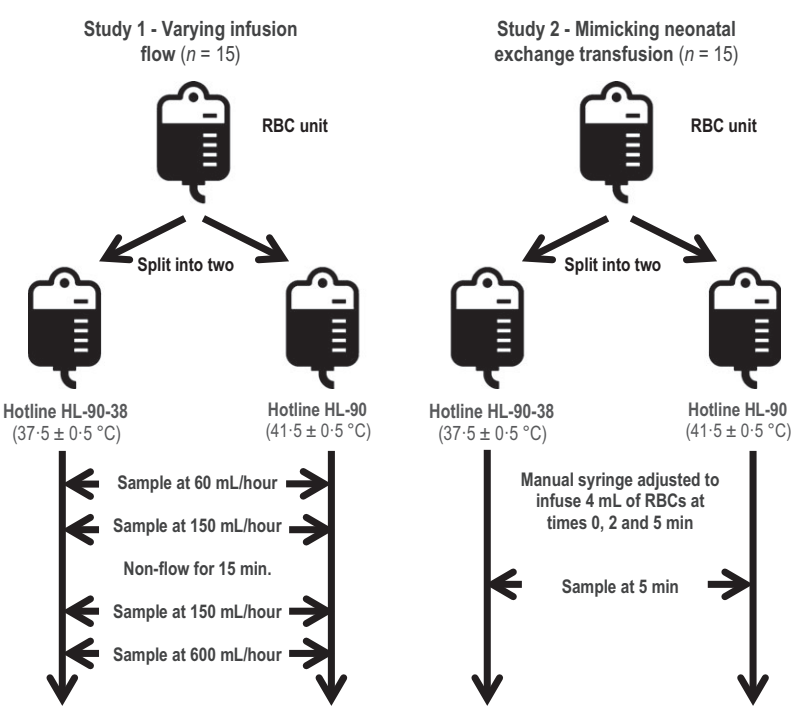

Fig. 1 Experimental design.

information on the RBC units was anonymous and did not allow identifying donors.

Results

Haemolysis

Variables collected did not follow a normal distribution. For both studies, a Wilcoxon signed-rank test for difference was therefore used and values for haemolysis are expressed by the median. The starting temperature of the RBC units to be warmed was about 4°C.

In the first study with the infusion pump, results in per cent haemolysis indicated no statistically significant difference between a warming at 37·5°C and another carried out at 41·5°C (Fig. 2). (Fig. S1 for FHb). Independently of the fluid warmer used, we observed high level of haemolysis at all flow rates and particularly at a flow rate of 600 ml/h where the haemolysis levels were well above the 0·8% or 1% regulatory requirements [1, 9, 10]. We also noted that the level of haemolysis decreased between 60 ml/h and 150 ml/h.

In the second study, which mimics neonatal exchange transfusion, no statistical difference was observed when RBC units were warmed at 37·5°C or 41·5°C (Fig. 3). (Fig. S2 for FHb). *P*-values for each comparison are given in supplementary Table S1.

Temperature profile

Figures 4 and 5 show the temperature profile of warmed RBC units. In each study, the room temperature was at 23°C. In study 1, the mean temperature of the blood products at the outlet of the tube is consistent with what was expected (Fig. 4). In study 2, when the blood product is aspired by a syringe, its temperature is about 1°C lower than expected (Fig. 5). This observation may be explained by a cooling of the blood product during the additional passage through the syringe, corresponding to a longer duration of flow. When the RBC unit remains stagnant for 2 min in the syringe, its temperature is reduced by an additional 1–2°C (–1·1°C for HL-90-38 and –1·5°C for HL-90). After 5 min of stagnation, the temperature loss is greater with an additional drop of about 2°C (–1·7°C for HL-90-38 and –2·2°C for HL-90). The difference between the temperature decline at 2 min and at 5 min is statistically significant for both blood warmers ($P < 0{\cdot}01$). The temperature profiles are presented in supplementary Table S2.

Modelling of factors that influence the level of haemolysis

Multivariate regression analyses were performed considering the various factors that may influence the level of haemolysis. The dependent variable was haemolysis in per cent. The choice of explanatory variables that may have an effect on haemolysis was based on major differences characterizing our tests as well as some of the

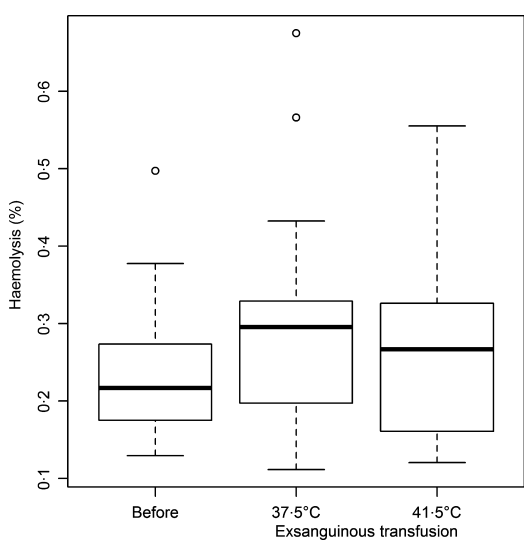

Fig. 3 Study 2 – Mimicking neonatal exchange transfusion. Box-plot of haemolysis (%) according to the blood warmer used.

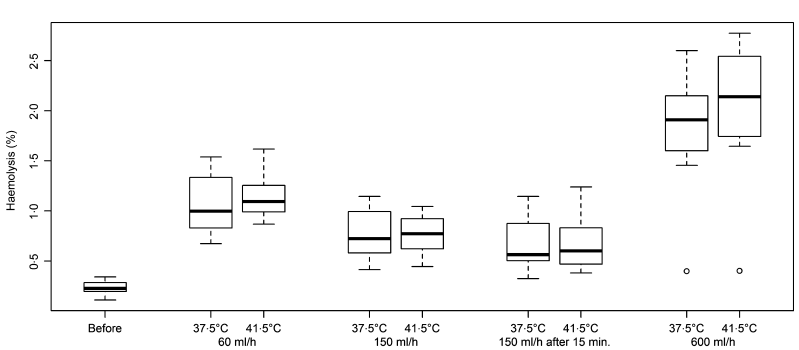

Fig. 2 Study 1 – Varying infusion flow. Box-plot of haemolysis (%) according to the blood warmer used.

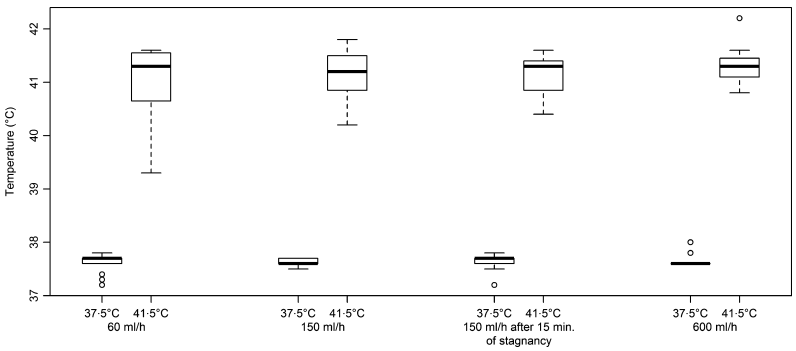

Fig. 4 Study 1 – Varying infusion flow. Box-plot of temperature according to the blood warmer used (room temperature at 23°C).

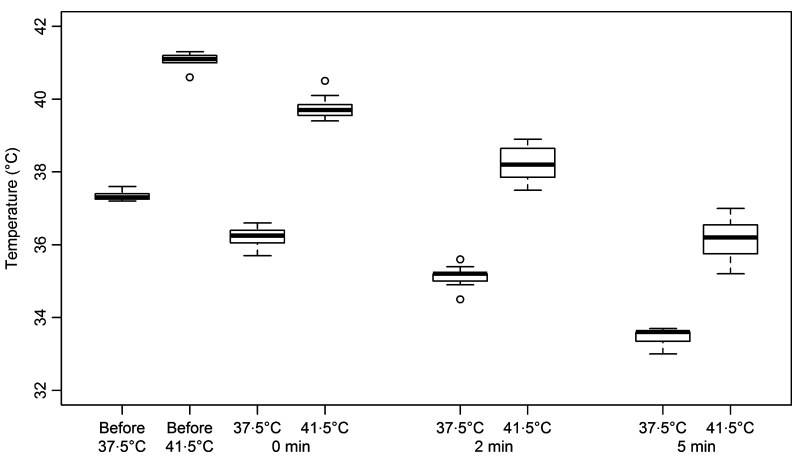

Fig. 5 Study 2 – Mimicking neonatal exchange transfusion. Box-plot of temperature according to the blood warmer used (room temperature at 23°C).

factors identified as such in the literature [5]. Explanatory variables selected on the basis of our tests were as follows: the use of an infusion pump and the flow rate, the use of a syringe mimicking an exchange transfusion in neonates, the type of blood warmer used and the stagnation time in the blood warmer. Factors selected on the basis of the scientific literature were as follows: the age of RBC units, the initial level of haemolysis before RBCs went through the blood warmer and the temperature of RBCs (i.e. the temperature at the outlet of the blood warmer). The level of haemolysis in blood units prior to flow through the fluid warmer was used to control the final level of haemolysis by its initial level. Some explanatory factors such as the concentration of CP2D and the delays between the warming experiments and the haemolysis assays were also tested without indicating statistically significant results. All the Shapiro–Wilk tests indicate a rejection of the normal distribution assumption for the variables used. Accordingly, multivariate regressions were corrected by the robust option to adjust standard deviations.

Estimates all have a coefficient of determination R^2 of 0·8 or more, which indicates that the model explains 80% or more of the variability of the response data around its mean, which is very high in estimates with individual data, indicating the good explanatory power of our models. The analysis of results presented in Table 1 shows five dominant factors to explain the final level of haemolysis following the flow of RBCs through the fluid warmers. These five factors are the initial level of haemolysis in the RBC unit, the use of the infusion pump, the age of the RBC unit and the flow rates at 150 and 600 ml/h. With the exception of the flow rate at 150 ml/h, all these factors strongly influence the increase of haemolysis in a statistically significant manner. These results also suggest that using an infusion pump produces an outcome two to three times larger than the initial level of RBC haemolysis and that this effect is almost doubled at the high flow rate of 600 ml/h. However, the use of a fluid warmer in conjunction with the use of an infusion pump does not generate additional haemolysis (i.e. *Blood warmer* × *Infusion pump* is insignificant in model 5). Regarding the significantly negative effect of the infusion pump at a flow rate of 150 ml/h, this is likely related to the fact that RBCs are subjected to considerably less pressure at 150 ml/h than at 600 ml/h. As regards to the age of the blood product, this variable has a significant influence and one additional day increases haemolysis by

0·024–0·027 points (i.e. an increase of 0·216–0·243 points from 30 days – youngest RBC in our study – to 39 days – oldest RBC). Considering that age of RBCs may be an important factor in determining haemolysis following warming, we checked this with the multiplicative terms *Blood warmer x Age of RBC*. This variable was positive but insignificant (model 4), thus indicating that warming older RBC up to 41·5°C does not generate additional haemolysis. Finally, the type of blood warmer used and the elevation of the warming temperature appear here to play only a limited role on the final level of haemolysis. Indeed, a blood warmer set at 41·5°C led to a small increase in the level of haemolysis compared to a blood warmer set at 37·5°C (i.e. a non-statistically significant contribution of 0·05 points percentage or 0·02 g/dl).

Discussion

The risk of haemolysis associated with the warming of RBCs during a transfusion is an important issue because of its potential negative consequences for the patient [3, 6]. Knowing the safe temperature level for warming RBC units during transfusion is therefore of utmost importance in transfusion practice. Several studies have tested the level of haemolysis associated with different warming temperatures using many types of blood warmers (water bath, countercurrent heat exchange, dry heat, chemical reaction, heating wire, microwave, radiofrequency) [5, 14]. Among these studies, a majority were published more than 20 years ago and only a few of them have studied commercially available blood warmers approved by health authorities [5]. Therefore, there is a growing interest in the transfusion community to evaluate the safety of current blood warmers because of the lack of published data. Previous studies have mostly reported the low risk of haemolysis when transfusing at a temperature of 37°C [15–21]. Beyond this temperature, the published results remain difficult to interpret and seemingly depend on the characteristics of the fluid warmer instrument, the stay duration of RBC in the warmer, the infusion pump type and flow rate, as well as the age of the blood product. Given these limitations and because transfusion practices (i.e. how we transfuse blood to patients) differ significantly from one institution to another, the present study was designed to evaluate the use of fluid warmers according to the transfusion practices of our institution either with an infusion pump at different flow rates or with a syringe for exchange transfusion in neonates. We therefore investigated whether the use of Hotline blood warmers commonly used by hospitals in Canada was safe when set to 41·5°C vs. 37·5°C with regards to the level of haemolysis.

Compared to previous studies investigating the effect of warming temperature higher than that of the human body on the risk of haemolysis [15, 22], our study is one of those with the highest numbers of RBC units tested

Table 1 Estimates of the effect of explanatory variables on the level of haemolysis

	Haemolysis (%)				
	M1	M2	M3	M4	M5
Initial value of haemolysis (before the test)	**1·222** (0·000)	**1·217** (0·000)	**1·201** (0·000)	**1·222** (0·000)	**1·222** (0·000)
Infusion pump (yes = 1; no = 0)	**0·831** (0·000)	**0·745** (0·000)	**0·436** (0·050)	**0·831** (0·000)	**0·795** (0·000)
Age of RBC unit (days)	**0·024** (0·028)	**0·025** (0·024)	**0·027** (0·018)	**0·024** (0·033)	**0·024** (0·028)
Blood warmer (HL-90-38 = 0; HL-90 = 1)	0·052 (0·285)	–	–	–	–
Temperature after blood warmer (°C)	–	0·020 (0·176)	0·094 (0·073)	–	–
Blood warmer × temperature after blood warmer	–	–	−0·007 (0·134)	–	–
Blood warmer × Age of RBC				0·001 (0·368)	–
Blood warmer x Infusion pump				–	0·072 (0·224)
Flow rate at 150 ml/h (yes = 1; no = 0)	**−0·350** (0·000)	**−0·352** (0·000)	**−0·358** (0·000)	**−0·350** (0·000)	**−0·350** (0·000)
Flow rate at 600 ml/h (yes = 1; no = 0)	**0·852** (0·000)	**0·848** (0·000)	**0·835** (0·000)	**0·852** (0·000)	**0·852** (0·000)
Stagnancy 15 min (yes = 1; no = 0)	−0·085 (0·082)	−0·085 (0·083)	−0·085 (0·083)	−0·085 (0·081)	−0·085 (0·084)
Constant	**−0·905** (0·016)	**−1·587** (0·011)	**−4·145** (0·032)	**−0·879** (0·020)	**−0·879** (0·020)
R^2	0·8040	0·8051	0·8068	0·8034	0·8048
Number of observations	150	150	150	150	150

Each model is labelled according to its order of appearance in the table (M1 to M5); explanatory variables are in the first column; the dependent variable is indicated in the first line; to determine the effect of each variable on the dependent variable, we need to multiply the coefficient in the table with the mean value of the explanatory variable considered; data in bold are significant at the 95% level. *P*-values are in parentheses. The number of observations corresponds to the total number of haemolysis assays (i.e. 120 in the experiment with the infusion pump and 30 with the syringe). Multiplying two explanatory variables in the regression model (e.g. blood warmer × infusion pump) is to test whether an interaction exists.

(n = 30). Furthermore, our study is the only one in this situation that has compared current fluid warmers approved by a health authority such as Health Canada or the FDA. Another strength of our study is that RBC units used for comparing the two blood warmers (HL-90 and HL-90-38) came from the same RBC unit (i.e. split RBC units) and that samples were collected before and after the blood heating by fluid warmers. Moreover, we used RBC units suitable for transfusion (i.e. not contaminated or outdated), which is different from earlier studies [18, 19, 23–26]. Unfortunately, our study is, however, limited by the fact that the effect of a blood warmer switched off on the level of haemolysis has not been investigated. Some studies have indeed indicated that the device configuration itself, without applying any warming, might affect RBC integrity and generate haemolysis [19, 27, 28]. This weakness, however, has only a little impact on the findings of our study, as our main objective was to determine the existence of a statistically significant difference in the level of haemolysis between two blood warmers set at two different temperatures. Moreover, in our second experiment with a syringe, RBCs flowed by gravity and as we found no additional haemolysis with a syringe, it can be concluded that the pressure exerted by the infusion tubing itself did not generate haemolysis. In addition, multivariate regressions allowed us to adjust for the major characteristics of the study and to isolate the impact of the blood warmer and its warming temperature. Our findings thus indicate that a blood warmer set at 41·5°C produces no more haemolysis than a blood warmer set at 37·5°C. Also note that our results are consistent with those reported in a recent systematic review with meta-analysis indicating that for a warming below 46°C, the increase in free haemoglobin level is not statistically significant and is in clinically negligible proportions [5].

Based on the comparison of our two series of tests (i.e. both using blood warmers, but one with an infusion pump and the other one with a syringe) and the results of our linear regression estimates (Table 1), it appears that the additional haemolysis generated by warming is quasi-null. By opposition, the per cent haemolysis of RBC is greatly increased by the use of an infusion pump, especially at high flow rates (600 ml/h). Indeed, we found that the final per cent haemolysis, as compared to its initial level of 0·22%, was multiplied by a factor of 3- or 4-folds at a flow rate of 150 (0·72 and 0·77% at 37·5°C and 41·5°C, respectively) or 60 ml/h (1·0 and 1·1% at 37·5°C and 41·5°C, respectively), which is close to or slightly exceeds the regulatory threshold of 0·8% in Canada. At a flow rate of 600 ml/h, the initial level was increased by more than eightfold (1·9 and 2·1% at 37·5°C and 41·5°C, respectively).

As regards to the level of haemolysis decreasing between 60 ml/h and 150 ml/h, this may be explained by the fact that each RBC unit was pumped using an infusion pump programmed with sequential flow rates, starting at 60 and 150 ml/h, followed by a 15-min stagnation period and resumed at flow rates of 150 ml/h and 600 ml/h. Considering the sequential nature of the test with the infusion pump, the blood product pumped at the beginning of the test was colder (starting temperature at about 4°C), showing more viscosity before to be warmed by the fluid warmer. As a consequence, the shear stress induced by the infusion pump may have been exacerbated by the natural resistance of a more viscous product (i.e. the ambient temperature in the room and the lag time between the refrigerator and the beginning of the test was not enough to make the blood product more fluid).

The Symbiq infusion pump used in our study uses a piston-actuated diaphragm cassette, and this device has not been specifically reported to generate RBC more haemolysis than peristaltic infusion pumps [3, 12, 29, 30]. However, considering these high levels of haemolysis using a blood warmer with the infusion pump Symbiq, we do not recommend to use a blood warmer with this specific model of infusion pump and to be cautious with the use of other infusion pumps. By cons, if the warming temperature had exacerbated the effect of the infusion pump on the haemolysis of RBC, we would have noted higher levels of haemolysis with the blood warmer set at 41·5°C, which was not the case. As a consequence, to our mind, the use of blood warmer is still suitable for blood transfusion. As regards to the age of the RBC units we used (30–39 days old), this one has a significant and non-negligible effect on haemolysis (0·216–0·243 percentage points) and it is possible that this have increased the effect of the infusion pump on haemolysis (i.e. older RBC are less resistant to shear stress).

In conclusion, during massive transfusion protocols or during blood exchange transfusion, the use of a fluid warmer adjusted to 41·5°C is probably the best choice for reducing the risk of hypothermia for the patient (i.e. the temperature was never below 35°C). As expected, the temperature of the blood warmer (37·5°C vs. 41·5°C) does not appear to affect the integrity of RBCs in routine infusion conditions prevailing in hospital settings. Further research is however required to ensure that the concomitant use of fluid warmers with high-speed pumps and other infusion pumps does not generate clinically significant levels of haemolysis. If not, it is worthy to determine the optimal conditions for an infusion pump to be used for the transfusion of RBCs.

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Authorship

DP and PB conceived the project. DP, JD and TGP designed the experiments. AJ and LT performed haemolysis measurements. SKB, DP and TGP were involved in the relation with the ethic committee. TGP and JFF provided statistical analysis. TGP and LT wrote the manuscript. All authors participated in the data analysis and revised the final manuscript. TGP is member of the FRQS funded Centre de recherche du CHUS.

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Conflict of interest

The authors declare no conflict of interests.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:
Fig. S1 Study 1 – Varying infusion flow. Box-plot of hemolysis (FHb in g/dl) according to the blood-warmer used.
Fig. S2 Study 2 – Mimicking neonatal exchange transfusion. Box-plot of hemolysis (FHb in g/dl) according to the blood-warmer used.
Table S1 Hemolysis results with regard to the blood warmer used.
Table S2. Temperature at the outlet of the blood warmer.?