Noninvasive pH Monitoring in Platelet Concentrates

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Keywords

Noninvasive pH measurement · Platelet concentrates · Donor screening

Summary

Background: New technology introduces the option to measure pH in platelet concentrates noninvasively. Findings that in one out of 25 apheresis concentrates the pH decreased significantly during storage motivated us to evaluate apheresis concentrates from 307 platelet donors. **Methods:** Small (15 ml) BSCI storage containers for noninvasive pH measurement were used in parallel with Fenwal PL2400 bags for storage of 5 days. Noninvasive pH measurement was compared to pH determination in samples from the storage container. Decrease of pH during storage was calculated as $\Delta\text{pH d2-d5}$. **Results:** The coefficient of correlation for noninvasive pH measurement ($n = 256$) versus standard methods was $R^2 = 0.964$ (pH electrode) or 0.952 (ABL 510). pH values in BSCI bags were slightly lower than in Fenwal PL2400 containers. Concentrates collected from 8 of the 307 donors showed a significant drop in pH. Repeated collection did not confirm these findings with a single exception. **Conclusion:** Noninvasive pH measurement demonstrates a high reproducibility. Our study could not confirm the frequency of 1 out of 25 plateletpheresis donors with concentrates developing a significant drop in pH during storage. This may be due to a substantially lower platelet concentration in our preparations.

Introduction

The pH value is established as quality parameter for stored platelet concentrates (PCs). pH values below 6.8 are associated with significant reduction of the in vivo survival of the stored platelets [1]. During storage, platelets generate metabolites, especially lactate which acidify the platelet concentrate. Correlation of pH decrease and morphological/physiological changes has been consistently demonstrated in several studies [2–5].

pH values have been determined in most studies by use of blood gas analyzers or pH electrodes. Both methods require sampling from PCs to perform the measurement. This sampling carries a substantial risk of contamination of the PC and is tedious, and, dependent on the numbers of samples to be collected, the unit may no longer be available for therapeutic purposes. In 2009 Reed and coworkers [6] described a method for noninvasive pH measurement with a fiber optic fluorescence detector (fig. 1). Based on the ability of certain organic compounds to change color with pH, conjugated fluorescent dyes were immobilized on a nitrocellulose membrane. The membrane was punched on a sensor disk and inserted into a tube with a clear polycarbonate window for fluorescent reading. Light emitted through the polycarbonate window generates a pH-dependent signal at 600 nm and a pH-independent signal at 568 nm. The calculated ratio of both signals represents the pH value.

In 2010 a study demonstrated that apheresis PCs from about 1 out of 25 donors developed a reproducible and significant drop in pH during their storage due to an increased glycolysis rate [7].

These findings and the availability of the noninvasive pH measurement method motivated us to perform a screening of PCs from donors in the Northwest of Germany. The aim of the study was to identify PCs with a significant pH decrease and to calculate their frequency in relation to the number of donors.

Fig. 1. Principle of noninvasive pH measurement (left) and image of pH1000 fluorescent reader (right).

Fig. 2. Noninvasive pH determination (BCSI) compared to standard methods ($n = 256$). A BCSI versus pH electrode (pHM220). B BCSI versus blood gas analyzer (ABL510).

The qualities of noninvasive pH measurements for PCs stored in 1,000-ml bags was demonstrated recently [8]. In this study we included small storage bags (15 ml) with integrated pH device as a new approach.

Material and Methods

Noninvasive pH Measurement

Comparison to Standard Methods

Samples from 64 buffy-coat-derived PCs stored for 8 days were used to compare the noninvasive measurements with standard methods (determination of pH at day 1, 5, 7, and 8). A 1-ml PC sample was used to determine the pH with the pH electrode M 220 and the blood gas analyzer ABL 510 (both Radiometer Inc., Copenhagen, Denmark). At the same time, the pH was measured noninvasively with the pH device of the bag and the fluorescence reader (BCSI pH1000; Blood Cell Storage Inc., Seattle, WA, USA) according to the manufacturer's instructions.

Reproducibility of Noninvasive Measurement

20 single-donor PCs stored for 7 days were used for repeated determination of pH with the noninvasive method. Each PC was examined three times a day with 6-fold repetitions, giving 420 data points for calculation of reproducibility. In addition 3 PCs with different length of storage were used for 18 repeat measurements.

Evaluation of 15-ml Bags

Apheresis PCs from 12 donors were stored in a Fenwal PL2410 bag (1,000 ml) for 7 days. A 12-ml aliquot from the same unit was stored in parallel in a BCSI bag (15 ml) with integrated device for noninvasive pH determinations. Noninvasive pH measurements were compared to pH determination (electrode M 220) in samples collected from the storage container.

PC Screening

Single donor platelets were separated with the AMICUS™ cell separator. Platelet in plasma suspensions were removed from the Amicus sampling bag and transferred into the small BCSI bag by sterile docking. For optimal storage conditions, the inner surface of the bag was covered with platelet suspension by filling the bag to maximum volume. After a resting time of 4 h the volume was adjusted to ± 13 ml. Platelets from 307 different donors were sampled and stored for 5 days. Noninvasive pH measurement was performed and the decrease of pH values from day 2 to day 5 of storage was calculated as $\Delta\text{pH d2-d5}$.

Statistics

Statistical analysis was done using DataDesk® Software version 6.2.1 (Data Description, Inc., New York, NY, USA). Significance of correlation was calculated using ANOVA (analysis of variance) and the Scheffé method for a single-step multiple comparison of dependent variants.

Fig. 3. Reproducibility of the noninvasive pH measurements calculated from 18 repeated pH measurements of 3 PCs ($\bar{x}$ = mean, SD = standard deviation; CV = coefficient of variation in %).

Results

Noninvasive pH Measurement

Comparison to Standard Methods

The storage of 64 PCs and four times determination of pH provided 256 data pairs for comparison of noninvasive pH measurement versus standard methods. pH values were distributed over a range of 6.40 to 7.51. Comparison of pH values measured with the pH1000 instrument and the pH-electrode M 220 gives a coefficient of correlation of $R^2 = 0.964$ (fig. 2a). A high correlation ($R^2 = 0.952$) was also observed for the data pairs of noninvasive pH values and the corresponding values from the blood gas analyzer (fig. 2b).

Reproducibility

A mean standard deviation was 0.01 (0–0.04), calculated from the 6-fold measurement of 420 samples in a pH range of 6.83–7.58 (data not shown). The coefficient of variation was 0.13%. No differences in reproducibility regarding lower versus higher pH values could be observed. The high reproducibility of the pH measurements was also demonstrated by the results from 18 repeat measurements of three different PCs (fig. 3), stored for 1 (unit 1), 5 (unit 2), or 7 (unit 3) days.

BCSI Bags (15 ml)

Mean pH values of 7-day storage Fenwal PL2410 bags versus BCSI bags are depicted in figure 4. Up to 5 days pH decrease for both bags show similar curve fittings, with slightly lower pH values for the 15-ml bags (difference 0.10–0.15). The extended storage time of 7 days significantly increased the pH decrease in the small bags ($p \leq 0.0001$; fig. 4).

PC Screening

Aliquots of apheresis PCs from 307 different donors (196 male, 111 female) were tested noninvasively for the pH decrease during storage. Separation of single donor platelets were performed for double dose concentrates ($n = 190$) or sin-

Fig. 4. Mean pH values of apheresis PCs stored in a Fenwal PL 2410 bag compared to 12 ml aliquots stored in 15 ml BCSI bags ($n = 12$).

gle units ($n = 117$). The mean volume of the small bags was 13.5 ± 1.2 ml with a platelet concentration of 978 ± 140 /nl. A pH value ≤ 7.00 after 24 h of storage was observed in 37/307 donors. The same samples reached expected pH values (7.22–7.71) after 48 h (fig. 5). For comparable results the pH decrease was calculated as Δ pH d2–d5. The median pH drop was 0.10 (range 0.01–0.32). Eight donors could be identified with significantly higher Δ pH values ($p \leq 0.0001$, fig. 6). For proof of reproducibility of the results a second donation of these donors were performed. Δ pH d2–d5 values of 6 donors appeared in the repetition as normal, with two of them near the highest connected data point (0.31). Only one donor shows also in the second collection a significantly higher pH decrease. One donor was excluded from repeat donation according to standard operation procedures.

Discussion

Noninvasive fluorescence measurements of pH offer an innovative tool to reliably monitor pH changes in PCs during stor-

Fig. 5. Noninvasive measurement performed in 15 ml BCSI bags (n = 307): the red symbols indicates unexpected low pH values measured at day 1 (left) and their normalization at day 2 (right).

Fig. 6. pH decrease during storage of 307 PCs calculated as $\Delta\text{pH d2-d5}$. Outlier are plotted with a circle, extreme values with a starburst.

donor ID	$\Delta\text{pH day 2 - day 5}$	
	first donation	second donation
80	0.63	0.31
39	0.52	0.08
219	0.42	0.38
260	0.41	n.d.
266	0.41	0.17
285	0.38	0.31
212	0.34	0.01
142	0.34	0.19

age. Direct comparison of noninvasive pH measurement to standard methods demonstrated the validity of the fluorescence method. Repeated noninvasive measurements with and without undocking the bag to the pH 1000 instrument showed the excellent reproducibility of this method ($\text{SD} \leq 0.03$).

The availability of 15-ml bags with integrated pH sensor offers the opportunity for pH screening in a small volume during storage of PCs. By sterile docking the 15-ml bag to the sampling bag of the cell separator the PC remains unattached and available for therapeutic treatments. For up to 5 days of storage, the pH values of noninvasive and standard methods are comparable except slightly lower pH values for noninvasive measurement in the small bag. In our opinion, these lower values are tolerable for a screening of PCs focused on the trend of pH changes. In contrast, the noninvasive pH de-

termination in large bags may be more useful in studies with the pH value as a mirror for the physiological conditions of stored platelets.

The handling of storage bags with integrated pH devices – independent of the bag volume – is easy and requires only few things to keep in mind. An incubation time of 4 h is necessary to wet the membrane of the pH device properly otherwise the measurements could be instable. In our study we used an incubation time of 4 h with the bag filled to his maximum to ensure the necessary conditions for the pH measurement and to cover the inner surface of the bag for a proper gas exchange. Despite these precautions the pH values measured after 24 h were in 12% of samples lower as expected and returned to a normal range at the 2nd day of storage. Air bubbles in front of the fluorescence detection window are a known interfer-

ence in noninvasive pH measurement. We assume that invisible air bubbles were the reason for the unexpected low pH values and that the bubbles dissolved during extended agitation of the bag. This may be critical if determination of pH values is planned as single point measurement after 24 h. To obtain comparable results in our study, the $\Delta\text{pH d2-d5}$ was calculated and used to identify donors with a significant pH decrease.

The published frequency of 1 out of 25 donors with a significant drop in pH during storage could not be confirmed in the screening of 307 donors from the Northwest of Germany. Eight donors with a noticeable decrease in pH were observed in the first donation, resulting in a frequency of 1 out of 38.5. Only one donor remained with a reproducible drop in pH after the repetition of the platelet collection, leading to an estimated frequency for the examined population of 1 out of 307. The platelet units for our donor screening were prepared according standard operation procedures with a resulting mean platelet count of 978/nl. Compared to the published data, the platelet concentration was less than 50% and could explain the failure to identify donors with a significant pH drop because of a higher lactate production.

Lactate overproduction observed in patients with myeloproliferative disorders was explained with modifications in the regulation of the enzyme glycogen phosphorylase [9]. Single nucleotide polymorphisms in genes coding for enzymes of glycolysis and lactate production with various distributions in different populations could be also an explanation for the higher lactate productions.

Preparation of platelet units according standard operation procedures and sterile docking of the satellite bag for noninvasive pH measurement should exclude the possibility of a bacterial contamination. Nevertheless, it is well known that bacterial metabolism can induce a significant drop in pH, and a check for sterility of the units used in this study was not performed.

The technique of noninvasive pH measurement is easy to perform and do not require any expertise in laboratory methods, offering the opportunity to include this method in the quality management of blood banks, e.g., as a final check of platelet units before transfusion. This could be done either in the blood bank with the small bag as backup sample or in the hospital with the PC in a storage bag with integrated pH device.

Further investigations on quality parameters of PCs and platelet function should help to further optimize the treatment of patients with PCs.

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Disclosure Statement

The authors declare that they have no conflicts of interest relevant to the manuscript submitted to TRANSFUSION MEDICINE AND HEMOTHERAPY.

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