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Ten Days Storage of Platelets: Analysis of Quality and Safety

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The necessity for Platelet Concentrates (PCs) in transfusion has increased, and several measures have been undertaken to try and increase the shelf-life of this product. Increasing the shelf-life to 10 days from the current 5 days will increase availability and reduce waste and cost. However, the PCs need to maintain their safety and therapeutic efficacy over the extended storage time. To ensure the safety of this product, the bag should be sterile, with low concentrations of cytokines to prevent transfusion reactions, and the platelet indices should be within the therapeutic range to ensure efficacy. The aim of this study is to determine whether PCs retain quality standards and thus, remain fit for transfusion after being stored for 10 days in SSP+ at 22±2°C with constant agitation.
Study Design: Qualitative study.
Place and Duration of Study: National Blood Transfusion Service Malta, between March 2021 and June 2022.
Methodology: In this study, 20 PCs were analysed by sampling on Day 5 and Day 10 of storage and tested for platelet count and indices, pH, sterility of the product and cytokine concentrations.
Results: The results obtained showed a significant differences in the platelet count and platelet indices and pH. The difference in cytokine concentration of Interleukin-6 (IL-6), Tumour necrosis factor-α (TNF—α) and transforming growth factor-β (TGF-β) were not found to be statistically

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different between Day 5 and Day 10. Furthermore, 1 PC unit was positive during testing for bacteria detection.
Conclusion: These results showed that the difference between Day 5 and Day 10 is not alarming, and further studies should be made to prolong the storage life to 10 days.

Keywords: Platelet Concentrates; prolonged shelf-life; quality; safety; cytokines.

1. INTRODUCTION

Approximately 2.1 million platelet concentrates (PCs) are transfused every year in Europe and the demand is incessantly on the rise, owing to the increased use of targeted therapy and increased life expectancy [1,2]. PCs are stored at 22 ±2^{o}C, with constant agitation in a platelet additive solution for a maximum of 5 days. Alternatively, the shelf-life can be extended up to 7 days in conjunction with more stringent sterility testing methods or pathogen reduction techniques to reduce the risk of bacterial transmission [3]. Given their short shelf-life, blood establishments may occasionally struggle to keep up with the ever-growing demand. Therefore, the availability of PCs can be improved either by recruiting more donors or by extending the shelf-life of this product. Recruiting more donors is difficult since blood donation is voluntary which helps to improve the safety of the product. Platelets have a physiological lifespan of 10 days and increasing the shelf-life might be feasible and as an added benefit this would also reduce waste and cost [4].

Two challenges need to be addressed when extending the shelf-life of PCs: the quality and safety of the product (Fig. 1). Maintaining the quality of the platelets is important to ensure that the products remain therapeutically effective [5]. The quality of the platelets is affected by platelet storage lesions (PSLs) which are morphological, functional, and metabolic changes in PC that develop during collection, processing, and storage [6]. The use of platelet additive solutions (PAS), gaseous permeable bags and constant agitation, are important actions taken to sustain the metabolism of cells and prevent platelet activation [7,8].

The European Directorate for the quality of Medicines and Healthcare (EDQM) recommends that increasing the PCs is only feasible with appropriate control of bacterial detection. However, there are other parameters, such as

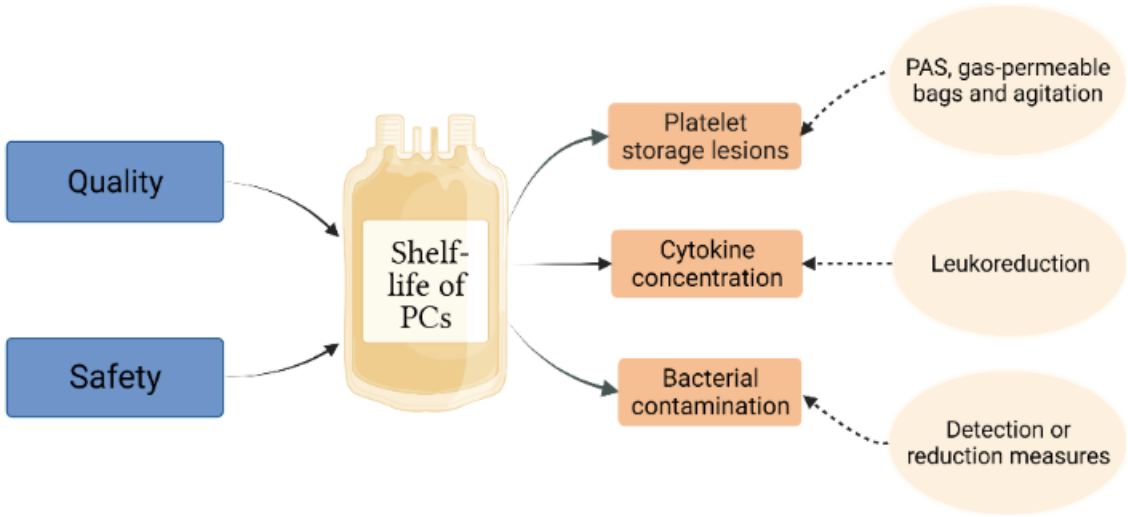

Fig. 1. The shelf-life of PCs is dependent on the quality and safety of Platelet concentrates. The quality of the PCs needs to be maintained by reducing platelet storage lesions. These have been combated with the use of platelet additive solution, gaseous permeable bags and agitation to maintain therapeutic efficiency. The safety of the products is ensured by looking at cytokine concentration and bacterial contamination to prevent transfusion transmitted reactions and infections. Cytokine concentrations mainly increases from white blood cells, and leukoreduction was found to decrease

cytokines, which need to be considered when ensuring the safety of the product during prolonged storage [3]. However other factors should also be considered in order to guarantee the safety of the products [9]. Cytokines in blood products can induce febrile non-haemolytic transfusion reactions (FNHTRs) or even more severe reactions such as transfusion-related acute lung injury (TRALI) and transfusion-related immunomodulation (TRIM) [8,10,11]. This study compared the platelet count and indices, variation in pH, as well as, the concentrations of cytokines; Interleukin-6 (IL-6), Tumour necrosis factor-alpha and tumour growth factor-beta on Day 5 to Day 10. In addition, sterility testing was performed on Day 10 to determine the risk of bacterial transmission.

2. MATERIALS AND METHODS

To determine whether the shelf-life of PCs can be extended to 10 days, a total of 20 expired PCs were retained by the National Blood Transfusion Service (NBTS), Malta. Out of the 20 PCs, 15 were random donor platelets (RDP) and 5 were single donor platelets (SDP). The RDPs were prepared from 5 whole blood donors with an identical ABO type at the NBTS laboratories, St Luke's Hospital, Malta. The SDPs were collected from a single donor via apheresis at the same centre. Ten of the units, 2 A-PCs and 8 BC-PCs, were irradiated at the NBTS laboratories. All PC units were stored in SSP+ (MacoPharma, Cat. No.: MASSP2030X) in gaseous exchange platelet pooling bags (MacoPharma Cat. No.: TRV8006XU) with continuous agitation (Helmer Scientific, United States) for 5 days at 22 ±2°C before analysis.

The weight, volume, platelet indices and quality control checks performed on Day 0 were obtained from the laboratory information system.

2.1 Sample Collection from Platelet Concentrates

On Day 5 the PC unit was weighed on an electronic balance scale (Precisa Gravimetrics AG, Switzerland), and the weight was recorded. To keep the integrity and sterility of the main platelet bag, approximately 5 – 6 mL of sample from the mother bag was transferred into a sample transfer bag (Fresenius Kabi Cat No.: A3AB0020) using the TSCD® II Sterile Tubing Welder (Terumo® BCT, United States of America). Prior to collection, the platelets in the mother bag were mixed by gently rotating the bag 10 times, then inverting and rotating the bags another 10 times, and finally mixing 10 times across to ensure a homogenous solution is obtained before transferring. The PC unit was then returned to the agitator at a controlled temperature of 22 ±2°C until Day 10, after which the sampling procedure was repeated.

2.2 Platelet Count and Indices

The Sysmex XN-550 automated haematology analyser (Sysmex Corporation, Kobe, Japan) was used to measure platelet count and indices to check the quality of platelets on Days 5 and 10. Platelet parameters, including platelet count, mean platelet volume (MPV), platelet distribution width (PDW), platelet large cell ratio (P-LCR), and plateletcrit (PCT), were analysed. In addition to the above, the quality parameters recommended by the EDQM included platelet content per final unit (10^{11}/L) and volume per $60x10^9$ of platelets (ml) were calculated.

2.3 pH assay

The pH was measured using a blood gas analyser (Radiometer, Copenhagen, Denmark on Day 5 and Day 10 at 22°C.

2.4 Inoculation and Processing of Culture Bottles

A pair of BPA (bioMérieux, Cat No.: 279044) and BPN (bioMérieux, Cat No.:279045) culture bottles were inoculated for every PC unit under investigation. The BPN and BPA bottles were loaded onto the Bact/ALERT® 3D system (bioMérieux, France) by scanning the sample barcode and entering the sample number manually. The bottles were incubated at a controlled temperature of 33 ± 2^oC for a maximum of 7 days. Bottles displaying a negative result were unloaded on the 7th day and checked visually one by one to ensure that no colour change was present. The culture bottles that were flagged as positive were removed upon detection irrespective of their incubation period. As for the negative culture bottles, these were also inspected visually to ensure a colour change was present. The positive samples were subcultured onto two Columbia blood agar plates (BioMérieux, Cat No.: 43050) and incubated respectively under aerobic and anaerobic conditions. When a pure culture plate was obtained this was sent to an external laboratory for identification.

2.5 Cytokine Concentration

A sandwich ELISA (R&D Systems®, United States) was used to analyse the concentrations of cytokines IL-6 (Cat. No: DY206-05), TGF-β (Cat. No: DY240-05) and TNF-α (Cat. No: DY210-05). The reagents were prepared in accordance with the Certificate of Analysis provided with each ELISA kit. Standard and sample analysis was carried out following the manufacturer's instructions. A microplate reader (Mithras, Berthold Technologies, German) was used to determine the optical density of each well.

2.6 Statistical Analysis

Statistical analysis was performed using IBM® SPSS® Statistics for Windows, Version 27.0 (IBM, United States). The Friedman test was used to compare data obtained from PCs on Days 0, 5 and 10. A p-value <0.05 was considered to be significant and was further investigated using pairwise comparison. The pairwise comparison determined where the difference occurred between the days. To investigate the difference in pH, Il-6 and TNF-α on Days 5 and 10, the Wilcoxon-signed ranked test was used. However, the paired sample T-test was used for investigating the difference in the concentration of TGF-β on Days 5 and 10. A p-value of <0.05 was considered a significant difference. All the results were expressed as mean values and 95% confidence intervals.

3. RESULTS AND DISCUSSION

A total of 20 PCs were analysed, and the data were statistically compared to determine if there is any significant difference in PCs stored on Days 0 (where applicable), 5 and 10. Due to the small sample size used in this study, the RDP and SDP were analysed together as a single cohort and the results are summarised in Table 1.

3.1 Platelet Count and Indices

The mean platelet count decreased from Day 0 (814.450 × 10^9/L) to Day 10 (736.850 × 10^9/L) and the difference was found to be statistically significant (*p*-value = 0.000). This was further investigated using the pairwise comparison. Fig. 2 shows that the difference was significant between all the days (p-value <0.05), but the most significant difference was between Day 0 and Day 10.

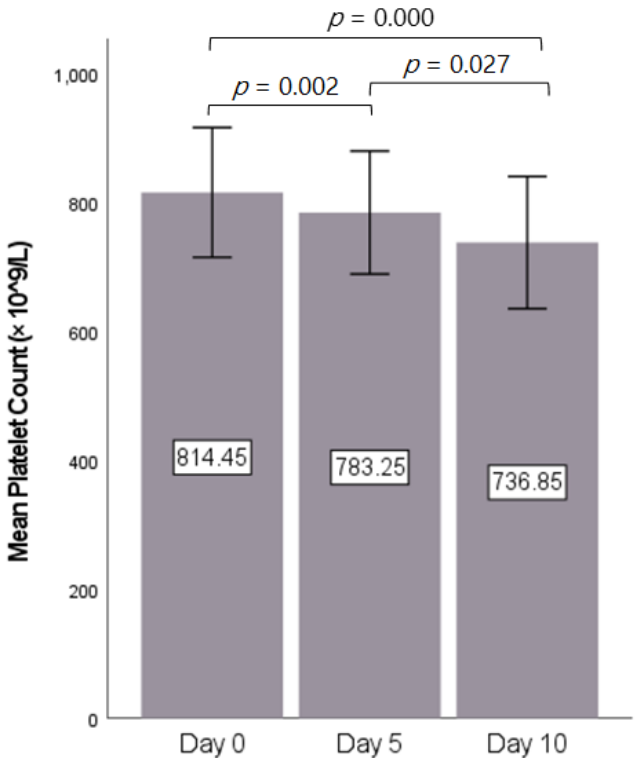

Fig. 2. Bar graph for the mean platelet count on Days 0, 5 and 10 of the 20 PCs. The error bars represent the 95% confidence interval (CI)

Table 1. Impact of extended storage on weight, platelet count, quality parameters and cytokine concentration in PCs at Days 0, 5 and 10 (n = 20)

Parameter	Day 0	Day 5	Day 10	*p*-value
Weight (gm)	413.90 ± 87.69 (254.00 – 486.00)	401.75 ± 87.90 (241.00 - 472.00)	388.05 ± 86.35 (234.00 - 460.00)	0.000
Platelet count	814.45 ± 214.86 (561.00 - 1170.00)	783.25 ± 203.44 (542.00 - 1119.00)	736.85 ± 218.58 (512.00 - 1130.00)	0.000
PDW (fL)	9.86 ± 0.49 (8.90 – 10.60)	9.50 ± 0.83 (8.40 - 11.70)	9.33 ± 0.78 (8.10 - 11.40)	0.003
MPV (fL)	9.74 ± 0.28 (9.10 - 10.20)	9.64 ± 0.47 (9.10 - 10.70)	9.62 ± 0.39 (9.10 - 10.50)	0.205
P-LCR (%)	21.02 ± 2.51 (16.10 – 25.00)	20.31 ± 4.08 (15.40 – 29.00)	20.09 ± 3.38 (15.40 – 27.90)	0.191
PCT (fL)	0.75 ± 0.21 (0.40 – 1.08)	0.74 ± 0.23 (0.36 – 1.19)	0.68 ± 0.23 (0.33 – 1.09)	0.012
PLT content per final unit (2×10^{11}/L)	2.71 ± 0.41 (2.27 - 3.82)	2.52 ± 0.438 (2.12 - 3.69)	2.24 ± 0.30 (2.01 - 2.90)	0.000
Volume per 6×10^9 of PLT	78.41 ± 19.30 (51.28 - 106.95)	81.41 ± 19.82 (53.62 – 110.70)	87.62 ± 22.45 (53.10 - 117.19)	0.000
pH	-	7.262 ± 0.088 (7.08 – 7.40)	7.097 ± 0.225 (6.54 – 7.31)	0.000
IL-6 (pg/mL)	-	34.32 ± 45.26 (6.21 – 192.12)	33.01 ± 44.57 (3.49 – 184.09)	0.681
TNF – α (pg/mL)	-	60.36 ± 62.50 (13.18 – 236.82)	59.16 ± 63.87 (13.18 – 236.36)	0.575
TGF-β (pg/mL)	-	108196 ± 16675 (81200.00 - 147200.00)	103240 ± 10787 (82933.33 - 121400.00)	0.122

Results are shown as mean ± SD (range)

The mean PDW on Days 0, 5 and 10 were 9.855 fL, 9.495 fL, and 9.330 fL, respectively, showing that the PDW decreased over the storage days with a statistically significant difference of 0.003 between the days. The difference was further investigated using the pairwise comparison and showed that the difference was significant between Day 0 and Day 5 (p-value = 0.009) and Day 0 and Day 10 (p-value=0.002) but not between Day 5 and Day 10 (p-value = 0.635) (Fig. 3).

The difference in MPV and P-LCR both reflect the size of the platelets but the difference in values over 10 days of storage were not found to be significant. Hence, a pairwise comparison was not performed.

PCT decreased with a 0.012 significance from Day 0 to Day 10, but when the pairwise comparison was performed, the difference was only found to be significant between Day 0 and Day 10 (*p*-value = 0.004).

The mean platelet content per final unit (x 10^{11}/L) decreased from 2.712 × 10^{11}/L on Day 0, to 2.243 × 10^{11}/L on 10 while the mean Volume per 60 × 10^{9} of Platelets increased from 78.414 mL on Day 0 to 87.621 mL on Day 10. The difference between the days for both quality parameters was found to be significant (*p*-value = 0.000). When a pairwise comparison was performed, the difference was found to be significant between all the days (Figs. 5 - 6), however, the values for all units remained above the cut-off values given by the EDQM [3].

3.2 pH Analysis

The pH decreased from 7.262 on Day 5 to 7.097 on Day 10 with a 0.000 significance, but the values of all units remained above the 6.4 cut-off value recommended by the EDQM [3].

3.3 Cytokine Concentration

Fig. 7 represents the bar graph for the cytokine concentration of IL-6, TNF-α and TGF-β. There was a slight decrease in all cytokines from Day 5 to Day 10 but the differences were not found to be significant.

3.4 Blood Cultures

Sterility testing on Day 10 for bacterial contamination showed one sample out of the 20 paired culture bottles to be positive. The organism was identified *as Cutibacterium acnes.*

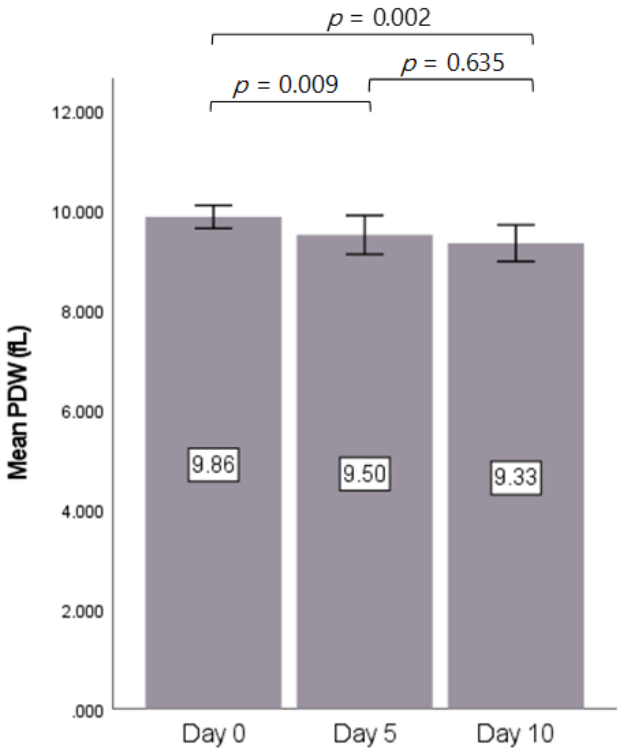

Fig. 3. Bar graph for the mean PDW on Days 0, 5 and 10 of the 20 PCs. The error bars represent the 95% CI

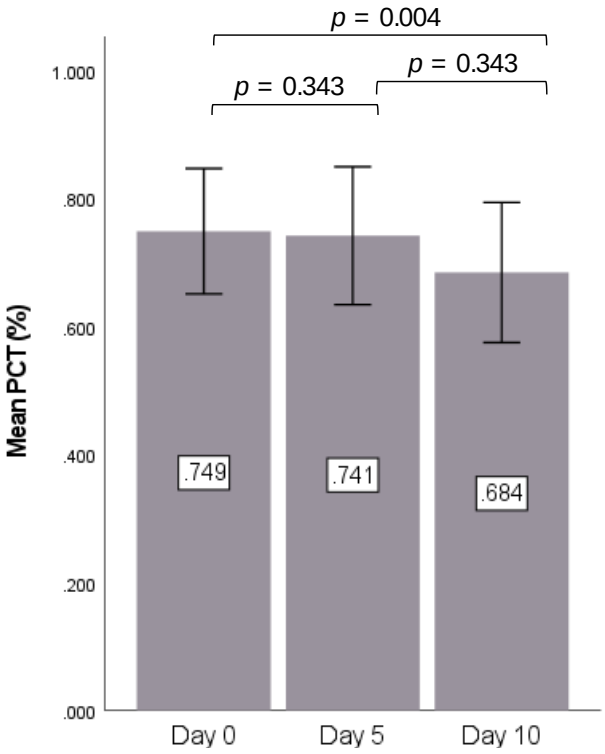

Fig. 4. Bar graph for the mean PCT on Days 0, 5 and 10 of the 20 PCs. The errors represent the 95% CI

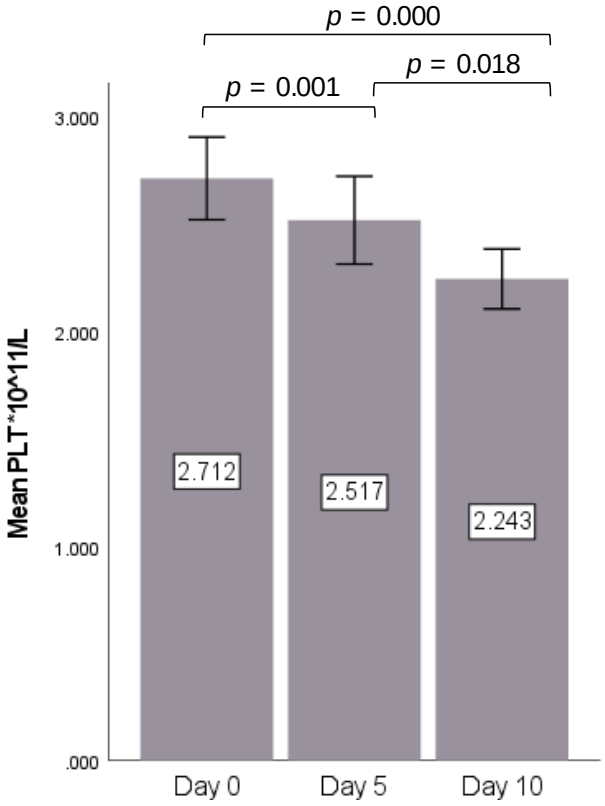

Fig. 5. Bar graph represents the mean platelet content per final unit (x 10^{11}/L) for Days 0, 5 and 10 of the 20PCs. The error bars represent the 95% CI

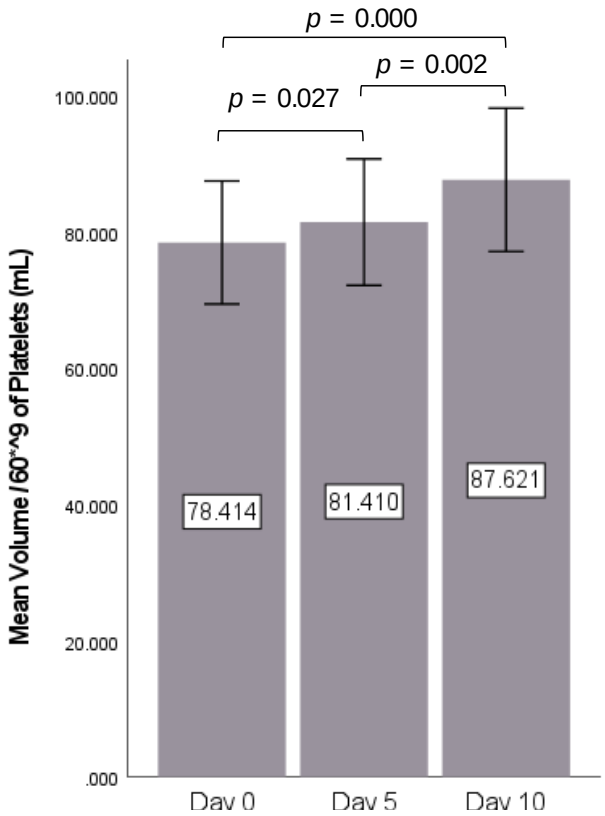

Fig. 6. Bar graph for the mean Volume per 60 × 10^9 of platelets (mL) on Days 0, 5, and 10 for the 20 PCs. The error bars represent the 95% CI

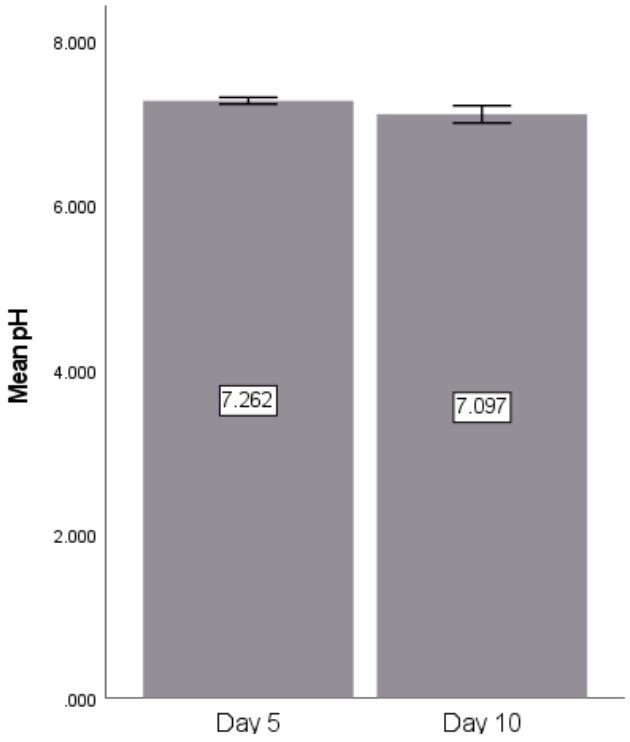

Fig. 7. Bar graph for the mean of the pH on Days 5 and 10 for 20 PCs. The error bars represent the 95% CI

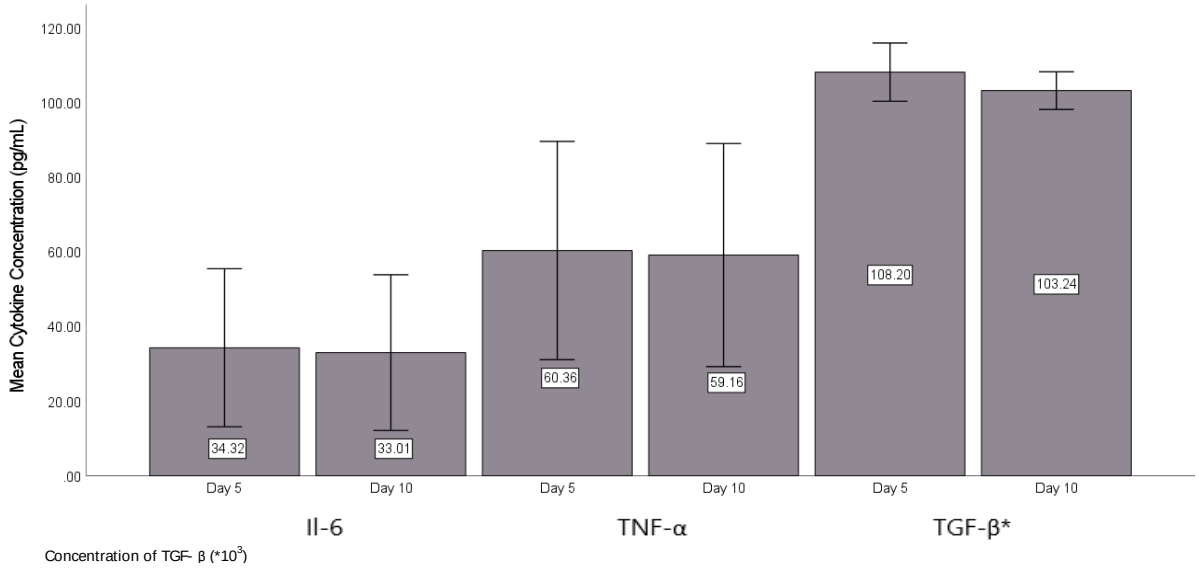

Fig. 8. Bar graph for the mean concentration of cytokines on Days 5 and 10 for 20 PCs. The graph shows the mean concentration of IL-6, TNF-α and TGF-β. The error bars represent the 95% CI

3.5 Discussion

Increasing the shelf-life of PCs will enable blood establishments to keep up with the demand and decrease wastage and cost. However, the shelf-life can be extended if the quality and safety are not compromised. In this study, the quality was assessed indirectly through platelet count, indices and quality parameters. The safety was analysed through sterility testing and by measuring the concentration of IL-6, TNF-α and TGF-β.

The platelet count ($x10^9$/L) and weight decreased significantly during the 10 days of storage and the difference was significant between all the days. However, when the platelet content per final unit ($x10^{11}$/L) was calculated from the platelet count and volume it remained above the cut-off value given by the EDQM (>2 × 10^{11}/L). This value decreased due to sampling on Day 5, which led to a decrease in weight and, consequently, a reduction in volume. This decrease in platelet count and platelet content per final unit was consistent with other studies [11–15].

The platelet indices, particularly the PDW and MPV, have become essential in assessing platelet function and morphology as they reflect the degree of PSLs [11,12,16]. The PDW demonstrates the variability in platelet size, while the MPV is a value calculated by the analyser that indicates the average size of the platelets [17]. In this study, there was a decrease in both values during the 10-days of storage. However, only the reduction in value between Day 0 and Day 10 for PDW was significant (Fig. 3). This decrease in PDW could be due to increased formation of microparticles and morphological changes in platelets during storage, both of which are a result of *in vitro* platelet activation. The mean P-LCR remained constant over the days while the PCT showed a significant decrease between Day 0 and Day 10 (p = 0.004). Given the circumstances, the results show that the difference between Day 5 and 10 for platelet indices is not statistically significant, indicating that the morphology of platelets was not affected to the extent that they would be inappropriate for transfusion purposes. Previous studies concerning platelet indices have produced contradictory results. For instance, Hornsey et al. [18] studied platelets obtained from BC-PC stored in SSP+ for 19 days and had complementary findings to this study with regards to the MPV until Day 12, but platelets began to increase in size after this day. The authors did not report any results on the values of PDW or other platelet indices over the storage days. Similarly, Singh et al. [12] showed that the MPV and PDW remained constant until the 7th day of storage. However, Bashir et al. [15] reported an increase in MPV over 10 days of storage from platelet-rich plasma (PRP) -PC and this correlated with another study by Chandra et al. [11]. On the contrary, Baghdadi et al. [16] reported that MPV and PDW decreased over 5

days of storage and this decrease was significant. The different results obtained from these studies could be due to the different PAS and pooling methods used.

The volume per 60 × 10^9 of platelets is another important quality parameter to ensure therapeutic efficiency, which needs to be above 40mL to maintain the pH of the PCs. This value significantly increased over the 10 days of storage due to a decrease in platelet count, which was not proportional to the decline in volume. Despite the statistically significant difference in values between the days for both parameters, the values obtained for all the units were still above the cut-off value (>40mL) given by the EDQM on Day 10 [3].

The pH fell from an average of 7.262 on Day 5 to 7.097 on Day 10 and the difference was found to be significant. However, the values of all units remained above 6.4 which is the cut-off value given by the EDQM [3]. A reduction in pH during storage was expected due to a decrease in volume after sampling and the production of lactate and other metabolites formed during platelet metabolism. However, this needs to be monitored as a pH below 6.4 can lead to irreversible morphological changes and platelet activation, making the PCs inapt for transfusion [5,19]. Several developments regarding PCs have been made to combat a decrease in pH; for instance, acetate and citrate are used as buffers in most PAS to maintain the pH. In addition, newer storage containers have been developed, such as Butyryl-n-Trihexyl Citrate (BTHC) and tri-(2-Ethylhexyl) trimellitate (TOTM), which support more gaseous exchange when compared to other plasticisers such as Polyvinylchloride (PVC) and Polyolefin (PE), which are essential for aerobic respiration and a decrease in lactate production [5,20–22]. Moreover, pH is used as an indicator for bacterial growth as bacteria produce by-products that are acidic, leading to a drop in pH [23]. For this reason, studies have also suggested the use of pH to monitor for bacterial contamination [24,25]. This decrease in pH over storage days is consistent with the literature available. Hornsey et al. [18] reported that the pH increased until Day 5 but decreased as glucose concentration declined and lactate concentrations increased. Saunders et al. [14] reported similar findings. Both studies utilized BC-PC stored in SSP+ and obtained values close to this study. However, other studies that used PRP-PC units and different PAS showed that the pH decreased during storage but decreased more significantly when the PC was stored in plasma [11,13]. Singh et al. [12] correlated the platelet indices and their effect on pH. They reported that indices such as PDW and P-LCR might be more valuable than platelet count alone to determine the changes in PC during storage. In addition to the metabolism of platelets and bacterial contamination, the presence of leucocytes also decreases the pH of the concentrates [26].

The results of the platelet indices and quality parameters indicate that the PCs maintained their overall therapeutic efficacy, and the difference between Day 5 and Day 10 is very minimal. This is owed to the replacement of plasma with SSP+. Several studies confirm that the use of PAS maintains the viability and quality of platelets for a longer time [13,15,26]. However, even though the platelet count and indices are useful in assessing therapeutic efficacy, further investigations are required to analyse their function as platelet indices do not correlate well with platelet aggregation tests as induced by adenosine diphosphate, collagen and epinephrine [27]. Moreover, several studies showed that increased storage time up to 7 days resulted in lower corrected count increment (CCI). Although the increase in storage time did not decrease morbidity or mortality, studies reported that the patients required platelet transfusions more frequently when older platelets were transfused due to an increased rate of *in vivo* clearance [28,29]. On the other hand, platelet function loss appears to e reversible upon infusion [30].

The environment where platelets are stored also provides a favourable environment for bacterial growth, and bacterial contamination is a major limitation when extending the shelf life. Bacterial contamination leads to an increased risk of septic transfusion reaction (STR) and transfusion-transmitted bacterial infections (TTBI) [31]. In this study, 20 pairs of culture bottles were inoculated on Day 10, and 1 BPN bottle was flagged positively after 5 days of incubation, and the bacteria were identified as *C. acnes*. The corresponding BPA bottle was not flagged positive. This organism is a common skin commensal and given that the environmental controls were negative. it was likely introduced from skin contamination during blood donation [32,33]. Recent studies reported that this organism was a frequent cause of positive blood cultures from PCs, and is commonly introduced during blood collection from the donor [34–37].

Pathogen reduction techniques such as the INTERCEPT blood system® and the Mirasol® PRT are already being utilised in some countries to inactivate bacteria and eliminate bacterial screening [33,38–41]. The benefits provided by these pathogen reduction methods include inactivating most bacteria and viruses as well as WBC, thus eliminating the need for bacterial screening and possibly leucoreduction leading to decreased risk of TTBI and STR without an increase in other transfusion-transmitted reactions (TTR) [42–44]. Despite these benefits, both the INTERCEPT blood system® and Mirasol® PRT have been associated with an increased rate in the production of PSLs in both BC-PC and A-PC [45–49]. This led to similar in vivo results with prolonged storage, i.e., decreased CCI and time interval between transfusions [44]. Therefore, other methods may need to be explored when prolonging the shelf-life of PC to keep a balance between safety and efficacy. For instance, large volume delayed sampling (LVDS) was found to be more appropriate when prolonging the storage life by up to 7 days and is more cost-effective than pathogen reduction methods [35,50,51]. Consequently, even though there is increased bacterial proliferation during prolonged storage, the risk of a TTBI or STR between Day 5 and Day 10 is analogous to the detection or reduction of bacterial contamination [36,52–54].

The risk of FNHTRs and allergic reactions such as TRALI and TRIM are among the common TTR associated with platelet transfusion [55–57]. Furthermore, these TTR were linked with cytokine production from metabolically active leucocytes that remained in the bag even after pre-storage leukoreduction [10,58–60]. Therefore, IL-6, TNF-α and TGF-β were monitored during storage on Day 5 and Day 10 to determine if they accumulate during extended storage. However, the concentrations of all the cytokines were found to remain consistent up to Day 10.

The results of previous studies on the concentration of IL-6 in PCs show that IL-6 concentrations in leucoreduced BC-PC or A-PC were below the detection limits or remained consistent up to 5 days of storage [10,55,59]. Costa et al. [61] monitored these levels over 9 days of storage and showed that IL-6 increased up to Day 7 but was consistent between Day 7 and Day 9; however, these changes were not statistically significant. The concentrations obtained from the study by Costa et al. [61] on leucoreduced BC-PC on days 7-9 were around 30 pg/mL, similar to those obtained from this study. Other studies have reported lower levels of IL-6 in leucoreduced BC-PC and A-PC on Day 5 of storage [10,26,58]. This difference may be owed to the difference in donor profile, collection and processing methods. In this study, the donors were kept pseudo-anonymised; therefore, no knowledge about age, gender or disease history was available. These donor factors may be significant because the levels of IL-6 may be higher in females, the elderly and individuals predisposed to chronic inflammation such as type 2 diabetes [62,63]. A more recent study by Kumar et al. [64] compared the levels of cytokines in non-filtered BC-PCs and filtered BC-PCs stored in SSP+ and A-PCs. They found that the levels of IL-6 were only detectable in non-filtered BC-PCs on Day 3 and Day 5. This further supports that the cytokine concentration increases during storage from the residual WBC. The undetectable levels in filtered BC-PC stored in SSP+ and A-PCs did not correlate with this study possibly due to different ELISA kits being used, with different sensitivities and specificities [64]. Ninkovic et al. [65] investigated IL-6 and IL-8 in BC-PCs and A-PCs and in recipients that had a transfusion reaction by monitoring their levels before and after transfusion. They found that the risk of FNHTRs with leucoreduced PCs increased if the recipients had previous inflammation, which resulted in increased IL-6 *in vivo*, and when combined with the transfused IL-6, it led to a concentration high enough to cause a TTR. Furthermore, they found no correlation between the age of the platelets and the risk of mortality, infection or thrombotic complication.

In this study, the mean concentration of TNF-α was 60.36 pg/mL on Day 5, which was reduced to 59.16 pg/mL on Day 10. This minimal change was not found to be statistically significant and correlates with the findings for IL-6 and that the risk of FNHTR on Day 5 and Day 10 from these products, with regards to these cytokines, is remarkably similar. However, these values did not correlate well with previous studies as they demonstrated that the concentration of TNF-α was exceptionally low or below detectable limits on Day 5 in leucoreduced A-PCs and BC-PCs [55,58,61,66,67]. Furthermore, Costa et al. [61] reported that the concentrations remained undetectable even after 9 days of storage. On the other hand, Wadhwa et al. [10] reported that the levels became detectable on Days 5 and 6 in PRP-PCs, with their results being similar to the concentration seen in this study (70-100 pg/mL).

However, they also analysed the biological activity of this cytokine and found it to be inactive. Consequently, further studies regarding the activity of TNF-α may need to be conducted to determine its role in FNHTRs and allergic reactions. Similarly, to the levels of IL-6, the levels of TNF-α may also change depending on the physiology, age, and sex of the individual [62,68]. Kumar et al.(64) found that the levels of TNF-α were undetectable in leucoreduced BC-PC but were detectable in A-PCs on Day 0 and that the levels decreased to an average of 6.1 pg/mL on Day 5, which is below the levels obtained with this study. As with the levels of IL-6, this could be due to the different ELISA kits used.

Numerous studies have shown that the levels of leucocyte-derived cytokines such as IL-6 and TNF-α are reduced significantly when the WBC count is reduced below 10^6 [55,58,64,66,67,69]. Chang et al. [55] reported consistent levels of these cytokines between Day 2 and 5 in pre-storage leucoreduced A-PCs, but the levels increased significantly in post-storage leucoreduced A-PCs. They reported that mild allergy reactions were more common in recipients transfused with the latter showing that these cytokines play a role in TTR. Depending on the donor profile, these cytokines may also be higher in concentration but may be reduced in PCs as the plasma is replaced with PAS [62,68,70]. The levels of these cytokines in leucoreduced A-PCs and BC-PCs may not affect otherwise healthy individuals but may be detrimental in patients with a weakened immune system. Nevertheless, since the difference in the levels of IL-6 and TNF-α on Days 5 and 10 were insignificant, the risk of FNHTRs and other allergic reactions remained consistent throughout the storage days.

The levels of TGF-β were reduced from Day 5 to Day 10, and the difference was not found to be significant. This analyte is a platelet-derived cytokine, therefore, leucoreduction will not decrease its level in PCs [6,10,55]. In contrast to this study, Wadhwa et al. [10] found that the levels of TGF-β increased over storage time in BC-PC and A-PC. The levels obtained were much higher for A-PC than BC-PC, but values were not as high as those obtained in this study.

The levels of TGF-β during storage reflect the degree of platelet activation as this is released from α-granules and may be used indirectly to monitor PSLs [10,71]. Consequently, the TGF-β concentration may be affected by factors that affect platelet activation, including collection, processing and storage methods [19,72]. For instance, Cardigan et al. [73] compared the levels of TGF-β using A-PC in different PAS and found that the concentrations increased up to Day 7, and the highest levels were obtained with 70% PAS-III (Intersol) modified. Seghatchian et al. [6] compared A-PCs obtained from six different apheresis machines and non-leucoreduced BC-PCs and found that the levels increased from day 1 to day 5 in all scenarios with the highest concentrations observed in platelets obtained by Amicus procedures. However, pre-storage or post-storage leucoreduction or irradiation of PCs did not affect the concentration of TGF-β as this was found to increase during storage, irrespective of the leucoreduction method used [55,74]. The consistent concentration of TGF-β in this study could be due to a consistent platelet activation rate during storage. The activation rate is likely to be higher during the collection and processing, but then it remains consistent or even reduces during storage [75–77]. Furthermore, Kim [78] correlated the levels of this cytokine in healthy blood donors with age and gender and found no significant difference between any groups. The role of TGF-β is multi-factorial, and its effects on transfusion and its relationship with transfusion reactions are difficult to predict [73,79]. However, this molecule plays a crucial role in the inflammatory response and may play a key role in TTR [79,80]. In addition, Labelle et al. [81] also reported that this marker, along with other growth factors, may increase the risk of metastasis.

3.6 Limitations and Further Studies

The inferences obtained from this study support the increase in storage time from 5 days to 10 days as platelets remain viable, and safety measures on Day 10 were found to be consistent with Day 5 of storage. However, the number of samples analysed was small, which might have hindered any differences between Day 5 and Day 10. The number of samples chosen was due to the limited production and funds available. In addition, ELISA is expensive, and funds were restricted to a single 96-microwell plate. Therefore, 20 samples were analysed as cytokine levels were measured in duplicates, and eight standards were used for each cytokine which included the blank. Moreover, the levels of cytokines may be affected by the donor's age, gender, physiology, and disease status [63], which could not be obtained for this study to

maintain the donor's anonymity. Furthermore, the cytokine concentration can also be affected by the limitations of ELISA. For instance, it only detects the quantity of the cytokine but does not reflect the biological activity and is greatly affected by the operator, manufacturer and environmental conditions. These limitations of ELISA should be considered in future studies for a more thorough assessment. In this study, the platelet activation was investigated indirectly through platelet indices and concentration of TGF-β, but further studies may use more direct platelet activation markers such as p-selectin and platelet function tests that reflect better the therapeutic efficacy of PCs [26].

Although this study indicated that the shelf-life of platelets could be increased to 10 days, further studies with a greater sample size would improve the accuracy of the statistical analysis. Furthermore, additional studies may wish to include an equal number of A-PCs and BC-PCs, providing further insight into the difference in PCs between collection methods. This was another limiting factor in this study. The levels of cytokines can also be investigated in terms of biological activity to confirm that their presence is a potential risk factor for recipients. Moreover, cold-stored platelets are regaining recognition since their halt in the 1970s as a cold temperature decreases the rate of metabolic activity of platelets and residual leucocytes which in turn, decreases PSLs and cytokine contamination making PCs safer while also maintaining their efficacy [82]. Cold temperatures also decrease the rate of bacterial proliferation, so the risk of TTBI and STRs may be similar to transfusion with red cell concentrates [82,83]. However, there is still debate whether the cold-stored platelets are as therapeutically adequate as PCs stored at 20-24^{o}C [83].

4. CONCLUSION

The significant differences obtained in this study were primarily due to the differences between Day 0 and 10 and not between Day 5 and 10. Therefore, therapeutic efficacy and transfusion risks remained comparable with extended storage, especially since at the end of storage, the quality parameters remained above the cut-off values given by the EDQM. Therefore, this study supports prolonging the shelf-life up to 10 days, but further research is required to ensure the therapeutic efficiency and safety of the product.

CONSENT

All participants where pseudo anonymous and couldn't be identified. Informed consent from the participants was granted during recruitment.

ETHICAL APPROVAL

Ethical approval (UREC FORM V_7606_24032021) for this study was obtained from the Faculty Research Ethical Committee, Faculty of Health Sciences, University of Malta.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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