Research Article

Synovial Fluid Cell Counts Remain Stable for 72 Hours Regardless of Storage Temperature: Implications for PJI Diagnosis

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Abstract

Background: Synovial fluid analysis plays a crucial role in the diagnosis of periprosthetic joint infection (PJI). However, the stability of leukocyte counts and the percentage of polymorphonuclear neutrophils (PMN%) under different storage conditions remains uncertain, and many institutions lack immediate access to on-site laboratories. We investigated whether storage temperature (room temperature (RT) vs 4 °C) influences synovial fluid white blood cell (WBC) count and PMN%, and if these parameters are stable for up to 72 hours after aspiration.

Methods: We prospectively analysed 106 synovial fluid samples obtained during revision arthroplasty for suspected PJI. Assuming that the population was homogenous according to the inclusion criteria, patient's samples were randomly allocated either to be stored at RT or at 4°C, in order to obtain two different set of samples. In both set of samples WBC count and PMN% were measured at baseline, 6, 12, 24, 48, and 72 hours using an automated haematology analyser after pre-treatment with hyaluronidase. Changes over time in the same patient synovial fluid and between different storage temperature groups were assessed with independent T test.

Results: Both WBC count and PMN% remained stable for up to 72 hours in samples stored at either RT or 4 °C. Mean WBC counts were slightly higher in refrigerated samples, but differences were minimal and not statistically significant. No variation led to reclassification of samples across the ICM 2018 diagnostic thresholds for PJI.

Conclusion: Synovial fluid WBC and PMN% remain stable for up to 72 hours regardless of storage temperature. These findings challenge the assumption that immediate analysis is required and support greater flexibility in clinical workflows, particularly in institutions without immediate on-site laboratory availability.

Introduction

Periprosthetic joint infection (PJI) is one of the most devastating complications of hip and knee arthroplasty, associated with high morbidity, mortality, and healthcare costs. Diagnosis remains challenging because symptoms are often subtle, and no single gold standard exists [1–3]. To address this, the 2018 International Consensus Meeting (ICM) incorporated synovial fluid white blood cell (WBC) count and polymorphonuclear neutrophil percentage (PMN%) as key minor diagnostic criteria [4-14]. These two parameters alone account for five of the six points required for achieving a definitive diagnosis and are also used to guide the timing of second stage reimplantation [15, 16]. Traditionally, WBC counts have required timely analysis, as delays or suboptimal storage conditions were thought to compromise accuracy [17–19]. To prevent protein precipitation, saline is often used as a diluent [20]. Early studies also described a rapid decline in WBCs after aspiration due to poor preservation, leading to their lysis. Conversely, subsequent reports indicated that storage at 4 °C might preserve cell counts for short periods [19, 21-23]. However, prior work has been limited to ≤24 hours or to comparisons of anticoagulants, leaving uncertainty about the stability of WBC and PMN% beyond the first day. The purpose of this study was to evaluate the effect of storage time (up to 72 hours) and temperature (room temperature (RT) vs 4 °C) on synovial fluid WBC count and PMN%. We aimed to determine whether delays in analysis alter these diagnostic parameters and affect classification according to the 2018 ICM thresholds for PJI.

Materials and Methods

One hundred and eleven consecutive patients with failed or painful joint arthroplasty of hip or knee seeking orthopaedic consultation between June and November 2023 were included. Inclusion criteria were age >18 years; diagnosis of PJI (knee or hip) based on ICM 2018 criteria and acute or delayed infection. The exclusion criteria were chronic inflammatory joint diseases; samples that were grossly haemolysed, or when the leftover was insufficient; antibiotic therapy not suspended at least 14 days before the procedure.

Residual samples from individual patients (106 patients) were used for the study, and a pseudo-anonymization protocol was applied immediately after routine examinations. All patients underwent a standardized diagnostic protocol, including clinical assessment and arthrocentesis; total WBC count and percentage of PMN were performed on SF ("best practice and according to the study protocol: T0). An experienced orthopaedic surgeon collected the SF during preoperative evaluation of joint disorder symptoms, suggesting a failure of uncertain origin of the knee prosthesis. Insufficient amount of SF (< 1.0 mL) was considered as an exclusion criterion. The SF was collected directly into K3EDTA tubes (Becton Dickinson, Franklin Lakes, NJ) and conveyed to the central laboratory within 2 hours at RT.

Automated leukocyte counting on SF was performed using a Sysmex XN-2000 (Sysmex, Inc. Kobe, Japan) equipped with a dedicated body fluid analysis module (XN-BF from Sysmex, Inc. Kobe, Japan). Specific quality control "XN check" (from Sysmex, Inc. Kobe, Japan) was used daily to assess imprecision and bias.

According to the laboratory consolidated routine, upon arrival at the laboratory, the SF was pre-treated a with hyaluronidase solution (Sigma Chemical Co., St. Louis, MO, USA), prepared by dissolving 2.5 mg hyaluronidase in 5 mL 0.1 mol/L phosphate-buffered saline at pH 7.4 (final concentration, 0.5 mg/mL). Pre-treatment consisted of adding 20 µL of hyaluronidase to 1 mL of SF, followed by incubation for 5 min at RT [24]. After routine analysis, the patients were divided into two groups and leftover samples were handled according to the following protocol: the first group of patients was maintained at RT, while the second was stored refrigerated at 2–8 °C. All the samples were then reanalyzed at specific time intervals, i.e., after 6, 12, 24, 48, 72 hours, i.e. T6, T12, T24, T48 and T72. Immediately after each measurement, the samples were returned to their original temperature conditions.

Statistical analysis

All statistical analyses were performed using IBM SPSS Statistics (version 29.0, IBM Corp., Armonk, NY, USA). Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were presented as frequencies and percentages. The Shapiro–Wilk test was used to assess the normality of continuous data distribution. Between-group comparisons of mean WBC counts and %PMN at each time point (baseline, 6, 12, 24, 48, and 72 hours) were performed using independent samples t-tests, with equality of variances assessed by Levene's test. Effect sizes were calculated using Cohen's d, Hedges' correction, and Glass's delta. Categorical variables based on diagnostic cut-off thresholds for WBC count and %PMN (according to the 2018 ICM criteria) were compared between storage conditions using the Pearson χ^2 test or Fisher's exact test, as appropriate. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using binary logistic regression to evaluate the association between storage condition and exceeding diagnostic cut-off values. A two-tailed p-value <0.05 was considered statistically significant.

Results

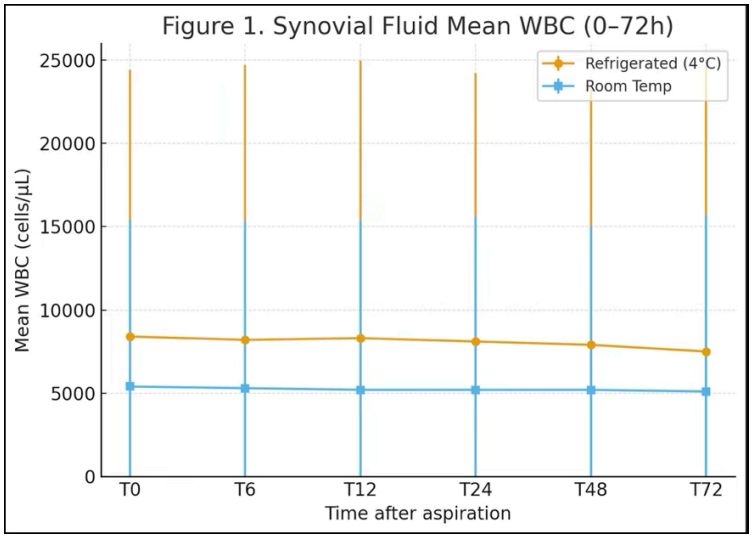
A total of 106 synovial fluid samples were analysed, with 52 stored at 4 °C and 54 kept at RT. All samples, as expected from the clinical situation, yielded a high number of WBC Across all subsequent time points (6, 12, 24, 48, and 72 h), WBC counts showed a progressive decline in both patients stored at Rt and at 4°C, with slightly higher values consistently observed in refrigerated samples, suggesting slightly better storage condition. Nevertheless, differences between storage conditions did not achieve statistical significance at any time point (p >

0.25 for all). In every patient, %PMN remained stable over time in both storage conditions, no statistically significant differences were found for %PMN at any time point ($p > 0.39$). A detailed summary of mean values, SD, and p-values for WBC and PMN% across all time points and storage conditions is provided in Table 1, complementing the graphical trends shown in Figures 1 and 2. Mean WBC values represent all samples assigned to each storage group at each time point (RT, n=54; 4 °C, n=52). Because each sample contributed to only one storage condition, per-sample trajectories between RT and 4 °C could not be shown.

Table 1: Synovial Fluid WBC and %PMN over Time (RT vs 4 °C).

Time	WBC RT (mean±SD)	WBC 4°C (mean±SD)	p-value WBC (RT vs 4°C)	PMN% RT (mean±SD)	PMN% 4°C (mean±SD)	p-value PMN (RT vs 4°C)
T0	5417.8 ± 10243.6	8496.4 ± 16712.1	0.94	49.8 ± 28.7	50.4 ± 28.8	0.87
T6	5334.6 ± 10241.2	8259.5 ± 16503.3	0.72	50.3 ± 27.2	51.8 ± 27.2	0.54
T12	5276.4 ± 10224.6	8340.4 ± 16647.5	0.81	51.2 ± 28.0	50.6 ± 28.3	0.61
T24	5327.7 ± 10416.0	8130.0 ± 16170.3	0.65	50.1 ± 28.0	53.9 ± 26.7	0.42
T48	5349.0 ± 10723.9	7884.2 ± 15622.3	0.33	50.2 ± 29.0	52.3 ± 27.2	0.21
T72	5176.5 ± 10628.5	7525.4 ± 15313.8	0.18	49.9 ± 28.1	56.6 ± 24.6	0.09

Figure 1: Mean (±SD) synovial fluid WBC counts over time in samples stored at room temperature or 4 °C.



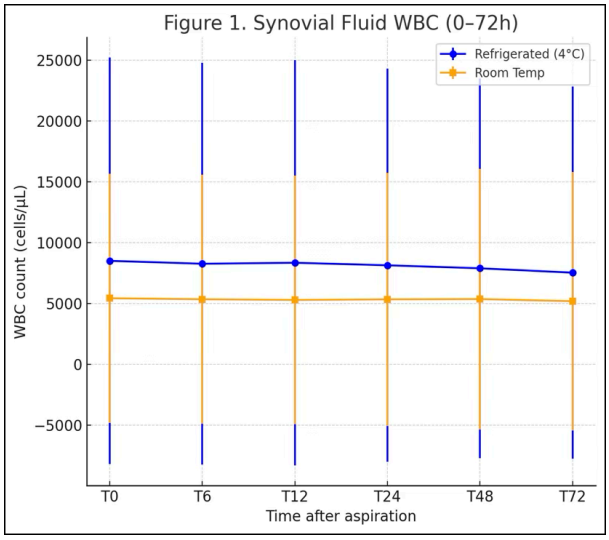
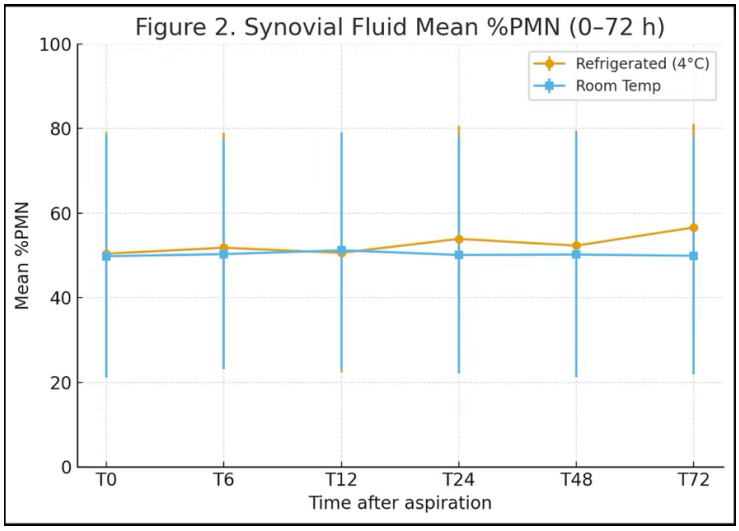
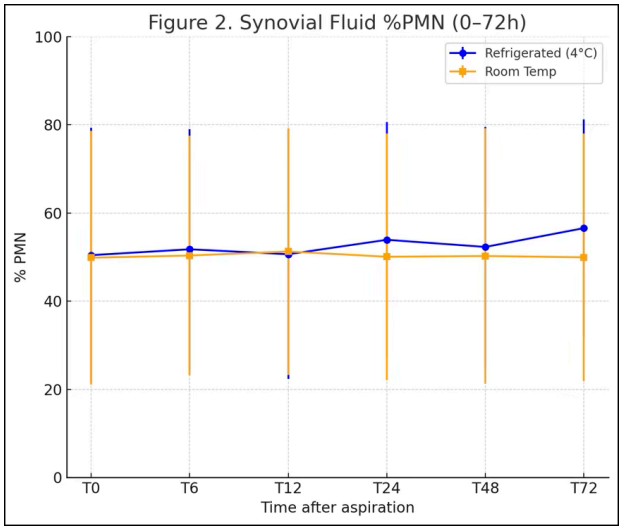


Figure 2: Mean (±SD) synovial fluid PMN% over time in samples stored at room temperature or 4 °C.





Effect size estimates (Cohen's d) for WBC counts ranged from 0.18 to 0.22 across time points, indicating minor, non-clinically relevant differences between groups. Effect sizes for %PMN were close to zero. Post-hoc power analysis was performed only for WBC counts, as this parameter highly exhibited distributions and very wide SDs. Using the observed absolute mean differences between storage conditions (≈2,300–3,100 cells/μL) and the pooled standard deviation at each time point, statistical power remained low (0.15–0.21), indicating that even moderate between-group differences would likely remain undetected. When applying the diagnostic cut-off values defined by the 2018 ICM criteria for periprosthetic joint infection, the proportion of samples above threshold was similar between groups at all time points. Pearson's χ^2 and Fisher's exact tests confirmed the lack of significant association between storage condition and exceeding the diagnostic cut-offs for either WBC count or %PMN ($p > 0.45$ for all). Binary logistic regression analyses showed no significant effect of storage temperature on the likelihood of exceeding WBC or %PMN diagnostic thresholds at any time point (all ORs close to 1, $p > 0.90$).

Discussion

Synovial fluid analysis has long been recognized as one of the most valuable diagnostic tests for PJI and for guiding the decision to proceed to a second-stage procedure in patients with a spacer [21]. Early evidence by Schumacher et al. showed that leukocyte counts may decrease as early as one hour after aspiration, leading the authors to consider synovial fluid analysis an emergency procedure [25]. However, subsequent studies have shown that reliable test results can still be obtained after 48–72 hours [26]. Manual cell count in synovial fluid has traditionally been considered the gold standard [22], but concerns about reproducibility and inter-observer variability have prompted attempts to automate the process. Vincent et al. [27] initially discouraged the use of automated counters due to artifacts such as fat droplets and cell damage. Conversely, Sugiuchi et al. [28] demonstrated that pre-treatment with hyaluronidase enabled the reliable and automated determination of leukocyte counts, with results comparable to those obtained through manual analysis. Although the limitations in obtaining hyaluronidase supplies in middle- and low-income countries need to be acknowledged, automated methods may offer advantages of higher precision, reproducibility, and time efficiency [22]. In our study, we employed automated leukocyte counting to evaluate the effect of storage temperature on synovial fluid WBC count and %PMN over 72 hours, comparing the effect of storing the same sample at 4 °C versus RT. Our findings indicate that refrigeration did not significantly influence either parameter at any time point, with both protocols showing similar temporal trends. Although mean WBC counts were generally higher in refrigerated samples, as predictable, these differences were minor and not statistically significant. This suggests that synovial fluid cellular parameters remain relatively stable over short-term storage, regardless of the temperature. When compared with earlier literature, some critical differences emerge. Koolvisoot et al. [29] first demonstrated that storage had a minimal impact on the accuracy of leukocyte counts, although their analysis was limited to short observation periods and manual methods. Later, Salinas et al. [26] investigated the role of anticoagulants and found that EDTA preserved leukocytes more effectively than heparin, even if a progressive decline in WBC could still be observed at 24 hours, emphasizing the importance of prompt processing.

In contrast, our 2023 data show that both WBC count and %PMN remain stable for up to 72 hours, independent of storage temperature, with no significant effect on ICM 2018 diagnostic thresholds. Taken together, these findings suggest a shift in perspective: while earlier studies highlighted the vulnerability of synovial samples to pre-analytical factors, our results support the view that synovial fluid may be less

vulnerable than previously assumed, and that short delays in processing, even up to three days, do not apparently compromise the diagnostic accuracy for PJI.
To this end, our findings extend current knowledge by demonstrating that short-term delays in synovial fluid analysis do not compromise the accuracy of WBC or %PMN measurements, regardless of whether samples are stored at RT or refrigerated. This challenges the traditional assumption that immediate analysis or mandatory refrigeration is required to preserve diagnostic reliability. From a clinical perspective, these results suggest that institutions without immediate access to on-site laboratories can safely delay the analysis up to 72 hours, thereby allowing for more flexible workflows without jeopardizing diagnostic accuracy for PJI.
Nevertheless, the absence of statistically significant differences should be interpreted with caution. Our study was not powered to detect minimal effects, and subtle changes in cellular morphology not reflected in quantitative counts may still occur. Furthermore, synovial WBC counts are highly heterogeneous, resulting in wide SDs. Across all timepoints, between-group differences were small (Cohen's d = 0.18–0.22), and post-hoc power analyses using observed mean differences and pooled SDs showed low statistical power (<0.30), indicating that subtle effects may have remained undetected. Although we complemented mean-based comparisons with categorical analyses aligned with ICM 2018 thresholds, this variability remains a limitation. Moreover, we focused exclusively on WBC and %PMN parameters, without evaluating other biomarkers (e.g., alpha-defensin, C-reactive protein) that may be more sensitive to storage-related degradation. Future studies should validate these findings in larger, multicenter cohorts using standardized protocols and incorporating a broader panel of synovial biomarkers. Such research could inform evidence-based recommendations for synovial fluid storage and transport, streamlining PJI diagnostic workflows. while maintaining accuracy.

Conclusion

Our findings indicate that the percentages of synovial fluid WBCs and PMNs remain stable for up to 72 hours, regardless of the storage temperature. This challenges the need for immediate analysis or mandatory refrigeration, suggesting that short delays do not significantly compromise the diagnostic accuracy for PJI. If confirmed by the stability of other parameters included in the Parvizi score, our results may help streamline workflows, especially in healthcare facilities without on-site laboratories.

Ethics Approval

The study was approved by the independent ethics committee on June 7, 2023. The research was conducted in accordance with the Declaration of Helsinki and national and institutional standards.

Conflict of interests

The authors declare no conflicts of interest for the conduction of the study.

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