Performance of 3 mL versus 5 mL Discarded Volume for Blood Sampling from Central Venous Access Device

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Background Central venous access devices (CVAD) are an essential part of safe practices in critical care, which enable effective venous access and help in avoiding repeated venipuncture. Discard method is widely practiced for blood sampling. A single occasion of blood sampling may cause minimal blood loss; however, the cumulative volume sequential sampling may become clinically significant. The study aims to reduce diagnostic blood loss, ensuring that the subsequent blood sample is not diluted or contaminated by residual intraluminal fluid.

Patients and Methods Within-subjects comparative design was adopted for 64 adult patients in the medical intensive care unit of a tertiary hospital. Two blood samples, using 3 mL and 5 mL discarded volume methods, were collected from each patient. Six serum parameters were measured on each of the paired samples and compared.

Statistical Analysis Used Paired t-test and Wilcoxon signed rank test were used for comparing the two methods. Bland–Altman plot analysis and intraclass correlation were used for clinically meaningful analysis.

Results When tested for fixed bias, there is no statistically significant difference between the methods. Potassium and creatinine levels showed significant proportional bias. The agreement limits of sodium, potassium, creatinine, and direct bilirubin were outside the clinically accepted interval, but the proportion of samples outside these intervals was less than 10%. All serum parameters showed excellent reliability, except for sodium which demonstrated good reliability.

Conclusions The practice of discarding 3 mL of blood for discard method is suggested, instead of the standard 5 mL to reduce iatrogenic blood loss. Thus, nurses in critical care are uniquely positioned to limit the diagnostic blood loss while obtaining blood samples.

Abstract

Keywords► blood discards
► discarded volume
► blood sampling
► critical care
► central lines
► central venous access device
► diagnostic blood loss

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Introduction

Central venous access devices (CVAD) are an essential part of safe practices in oncology and critically care. When not in use, they are flushed with heparinized saline to clear the lumen of blood. To ensure that the blood sample is not diluted or contaminated with the residual heparinized saline or fluid, the first blood is usually not sent to the laboratory.1

Different methods of blood sampling, including discard method, push–pull method, reinfusion method, and dead space method, have been reported to have their own advantages and potential complications.2 Discard method is widely practiced worldwide and is considered reliable. The discarded volume before blood sampling is likely to be determined by the size and type of the CVAD.3 However, in standard practice, a discard blood volume of 5 mL, greatly in excess of the estimated intraluminal volume, is generally recommended for all lines.3

A single occasion of blood sampling may cause minimal blood loss; however, cumulative volume of discarded blood from sequential sampling may become clinically significant. Dech and Szaflarski reported a mean blood loss of 18 to 377 mL/d in critically ill patients; discard volumes accounted for 24 to 30% of this blood loss.2 It was calculated that during the average intensive care unit stay of 3.5 days, 140 mL of blood is lost for laboratory purposes.5

There is no clear consensus about the volume of blood that needs to be discarded from a CVAD before sampling. Different studies recommend different discarded blood volumes, such as 6 mL for tunneled catheter, 9 mL for nontunneled catheter,4 25 mL for coagulation profile,5 5 mL for blood culture,6 and 4 mL for discarded volume.7 Thus, the practice of discarded volume widely varies and a proper method needs to be ascertained. The current comparison between 3 mL and 5 mL discarded volume aims to reduce unnecessary blood loss, decrease potential for infection and reinfusion of blood clots, as well as ensure that blood sample taken from CVADs will not be diluted or contaminated by the residual intraluminal fluid.

Methods

A within-subjects comparative study was conducted among patients admitted in a medical intensive care unit (ICU) with CVAD. The inclusion criteria were participants with age > 18 years and those with triple lumen central venous catheter (Seven French size, 15 cm). Patients with malfunctioning CVADs (without free flow) and patients who are severely anaemic (haemoglobin < 5 gm%) were excluded from the study. Consecutive sampling was adopted during the study period.

Objective: To assess the performance of 3 mL versus 5 mL discard volume methods of blood sampling from CVAD, done for the measurement of serum parameters.

Sample size calculation: For sample size calculation, sodium levels were used, as it had the broadest reference range among the measured parameters. Bland–Altman sample size estimation method was used.10 Using this method, to detect a mean difference of 2 units in serum sodium (with standard deviation = 0.5) with a two-sided significance level of 0.008 (Bonferroni correction for six outcomes), power of 90%, and a maximum allowed difference between the two methods as 3.4, the study required 64 participants.

Data collection procedure: Data was collected by taking two blood samples from each patient, using strict aseptic technique as per the Infusion Nursing Society guidelines.3 After aspirating and discarding 3 mL of blood, another 2 mL of blood was withdrawn with a new 10 mL syringe, transferred to a vial, and labeled as the first study sample. Another 10 mL syringe was taken to withdraw 2 mL (considering the 3 mL discarded volume and 2 mL first study sample as making 5 mL discarded volume), which was transferred to another vial and labeled as the second study sample. Thus, the total sample volume withdrawn for the study was 7 mL. Blood samples with proper labels were analyzed in a biochemistry laboratory. Six serum parameters (serum sodium, potassium, calcium, creatinine, total bilirubin, and direct bilirubin) were measured on each of the paired samples. Sample collection coincided with the taking of clinically indicated blood samples.

For 20% of the samples (13 samples), the test was repeated using the same sample on the same day and on the consecutive day to assess the reliability. Separated plasma after centrifuge was stored in a refrigerator (2–8°C for intraday reanalysis; −20°C for interday reanalysis).

Data analysis: The distribution of categorical variables such as gender, diagnosis, and site of CVAD placement were expressed as frequency and percentages. Continuous variables such as serum parameters (sodium, potassium, calcium, creatinine, and total and direct bilirubin) were expressed as mean with standard deviation (SD) or median with range. The comparison of serum parameters was performed by using paired t-test (normal distribution for sodium level) and Wilcoxon signed rank test (non-normal distribution for potassium, calcium, creatinine, and total and direct bilirubin). There was concern about using only paired t-test and Wilcoxon signed rank test comparison methods; often, the differences may be statistically significant yet not clinically meaningful. Therefore, Bland–Altman plot analysis11-13 was also used, which is the preferred approach for providing a clinically meaningful understanding of the differences between the two sampling methods. The reliability of serum parameters was assessed using intraclass correlation (ICC).14,15 All the statistical analyses were performed at 5% level of significance, and p < 0.05 was considered as significant.

Ethical approval: Institutional Ethics Committee (IEC) for human studies and Nursing Research Monitoring Committee (NRMC) approved the study. Informed consent was taken from patients or legally authorized representatives.

Results

Of the 64 participants with diseases involving almost all body systems, neurological (21.9%) and genitourinary systems (18.7%) constituted a larger proportion. Of the total, 56.3% patients were male and their median age was 46.5 years (►Table 1).

Comparison of serum parameters obtained by 3 mL and 5 mL discarded methods was performed by using paired t-test (normal distribution for sodium level) and Wilcoxon signed
rank test (non-normal distribution for potassium, calcium, creatinine, and total and direct bilirubin). Table 2 reveals that there is no statistically significant difference in the levels of serum sodium, potassium, calcium, creatinine, total bilirubin, and direct bilirubin between the 3 mL and 5 mL discarded volumes.

**Table 1** Baseline demographic and clinical variables of participants

| Participant characteristics (N = 64) |  |
|------------------------------------|--|
| Age (in years) median (IQR)         | 46.5 (29.5, 56.8) |
| Gender: Male n (%)                 | 36 (56.3) |
| Duration of illness (in days) median (IQR) | 10 (6, 17) |
| Duration of hospital stay (in days) median (IQR) | 6 (3, 9) |
| Site of CVAD placement: Jugular vein n (%) | 51 (79.7) |
| Duration of CVAD insertion (in days) median (IQR) | 4 (3, 6) |

Abbreviations: CVAD, central venous access device; IQR, interquartile range.

**Table 2** Comparison of serum parameters obtained by 3 mL and 5 mL discarded volume methods

| Parameters       | Discarded blood volume mean (SD) or median (IQR) | p value<sup>a</sup> |
|------------------|-----------------------------------------------|---------------------|
| Sodium (mEq/L)   | 135.7 (8.2) 136.1 (8.1) | 0.369 |
| Potassium (mEq/L)| 3.9 (3.5, 5) 3.9 (3.5, 5.1) | 0.295 |
| Calcium (mg/dL)  | 7.9 (7.5, 8.6) 7.9 (7.5, 8.6) | 0.119 |
| Creatinine (mg/dL)| 1.1 (0.7, 3.5) 1.1 (0.6, 3.4) | 0.470 |
| Total bilirubin (mg/dL) | 0.7 (0.4, 1.1) 0.7 (0.5, 1.1) | 0.197 |
| Direct bilirubin (mg/dL) | 0.2 (0.1, 0.6) 0.2 (0.1, 0.6) | 0.284 |

Abbreviations: IQR, interquartile range; SD, standard deviation.

<sup>a</sup>Paired t-test or Wilcoxon signed rank test.

There was concern about using only paired t-test and Wilcoxon signed rank test comparison methods; often, the differences may be statistically significant yet not clinically meaningful. Therefore, agreement between the two methods is tested using the Bland–Altman plots along with a clinically accepted interval (CAI) estimated a priori and based on clinical expertise. Bland–Altman plots (Fig. 1) show very small mean biases, and since the line of equity is within the confidence interval (CI) for mean difference, there is no systematic difference between two discarded methods. The agreement limits for calcium and total bilirubin levels were within the CAI (Table 3). The proportion of samples outside the CAI for the other parameters is also less than 10%.

Reliability of serum parameters was assessed for 13 samples using the ICC for continuous data, where ICC values less than 0.5, between 0.5 and 0.75, between 0.75 and 0.9, and greater than 0.9 indicate poor, moderate, good, and excellent agreement, respectively (Table 4).

All serum parameters, including potassium, calcium, creatinine, and total and direct bilirubin, showed excellent reliability (ICC > 0.9), except sodium, which has good reliability.

**Table 3** Descriptive data and Bland–Altman analysis data showing mean difference between findings obtained by 3 mL and 5 mL discarded sampling methods

| Parameters       | Reference range | Bias Mean bias | T stat | p value<sup>a</sup> | Proportional bias Regression coefficient | p value<sup>b</sup> | Limits of agreement (BA plot) | CAI | N | Values outside CAI range |
|------------------|-----------------|---------------|-------|---------------------|----------------------------------------|-------------------|----------------------------|-----|---|--------------------------|
| Sodium (mEq/L)   | 135–145 mEq/L   | −0.31         | −0.22 | 0.369               | 0.02                                  | 0.656            | 5.11 to −5.71               | −4.0 to 4.0 | 64 | 4 (6.2) (2.5–15.0%)      |
| Potassium (mEq/L)| 3.5–5.5 mEq/L   | −0.02         | −0.13 | 0.295               | −0.078                                | 0.001<sup>*</sup> | 0.38 to −0.43               | −0.4 to 0.4 | 64 | 4 (6.2) (2.5–15.0%)      |
| Calcium (mg/dL)  | 8.8–10.6 mg/dL  | −0.02         | −0.11 | 0.119               | −0.015                                | 0.582            | 0.47 to −0.51               | −1.0 to 1.0 | 64 | 1 (1.6) (0.3–8.3%)       |
| Creatinine (mg/dL)| 0.5–1.2 mg/dL  | −0.03         | −0.07 | 0.470               | −0.050                                | 0.000<sup>*</sup> | 0.43 to −0.50               | −0.3 to 0.3 | 64 | 5 (7.8) (3.4–17.0%)      |
| Direct bilirubin | 0–0.2 mg/dL     | 0.01          | 0.01  | 0.196               | −0.017                                | 0.067            | 0.23 to −0.21               | −0.1 to 0.1 | 64 | 4 (6.6) (2.5–15.0%)      |
| Total bilirubin  | 0.3–1.2 mg/dL   | −0.01         | 0.05  | 0.284               | 0.001                                 | 0.678            | 0.12 to −0.14               | −0.2 to 0.2 | 64 | 2 (3.1) (0.9–10.7%)      |

Abbreviations: 95% CI, 95% confidence interval for percentage of values outside the CAI range; BA plot, Bland–Altman plot; CAI, clinically accepted interval; n (%), number of samples with difference between the two methods outside the clinically accepted interval with percentage; N, number of samples tested.

<sup>a</sup>paired t-test.

<sup>b</sup>Analysis of variance (ANOVA).
(ICC = 0.75–0.9) at all time points, that is, at 0 hour, 6 hours, and 24 hours.

**Discussion**

When the blood sampling is taken for clinical or research purpose, it is recommended to remove an indicated volume of blood from a CVAD before a blood sample is obtained. This discard volume is considered to confirm that the blood sample obtained is not diluted with flushing solutions, thereby the results of the clinical parameters are not deranged. While this guarantees that the blood sample taken following the discard withdrawal will give a valid result, a volume of 5 mL discard is considered to be in excess of the intraluminal volume. Further, when this excess volume is taken on multiple occasions, especially in patients who require blood

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**Fig. 1** Bland–Altman plots for serum parameters with difference between 3 mL and 5 mL discarded samples plotted against the mean of the values. Green dotted lines, upper limit of agreement (ULA), and lower limit of agreement (LLA) refer to the limits corresponding to the two standard deviations (SD) from mean difference; blue dashed lines refer to the upper and lower limits of the confidence interval (CI) of the mean difference; the red solid line refers to the mean bias; and the black solid line refers to the line of equity.
transfusions such as in the cases of carcinoma, anemia, etc., it may be clinically significant.

Different methods were used for obtaining blood samples via CVADs—discard, push–pull, reinfusion, and dead space method—without clear consensus as to which is the most appropriate, effective, or safe method. Discard method is most commonly used while the recommended discarded volume widely varies. Thus, comparison was done between the 3 mL and 5 mL discarded volume methods for the serum parameters—sodium, potassium, calcium, creatinine, and total and direct bilirubin.

The result of comparison between 3 mL and 5 mL discarded volumes shows that there is no significant difference in the serum levels. The finding is also supported by a Cole et al study which was conducted in two parts—central venous lines (CVL) and Portacaths. In CVL (3 mL vs. 5 mL), the effect of discard volume on mean analyte levels was negligible, except for total protein (mean difference in total protein was 1%, 95% CI). In the Portacath study (5 mL vs. 2 mL, 3 mL, 4 mL, and 5 mL discarded volumes), the result indicates that there is lesser difference in the 3 mL and 5 mL discarded volumes.

While assessing the agreement between 3 mL and 5 mL discarded volume methods for the six parameters, the Bland–Altman agreement limits for sodium, potassium, creatinine, and direct bilirubin were not found within the CAI (as defined a priori and based on clinical expertise). The percentage of samples resulting in such a deviation for sodium, potassium, and direct bilirubin are 6.25% (95% CI 2.46–15%), and for creatinine it is 7.81% (95% CI 3.38–17%), which is considered small and clinically insignificant as the deviation is found in less than 10% of the samples. Overall, the 3 mL and 5 mL discarded volume methods are considered to be in agreement. This is in contrast to the findings of Cole et al, where calcium levels from the 5 mL discarded volume samples were significantly different than the 2 mL or 3 mL discarded volume samples. However, it was not considered clinically significant in that study.

The test–retest reliability of serum parameters at the initial time point (0 hour), after 6 hours, and after 24 hours is based on ICC. The potassium, calcium, creatinine, and total and direct bilirubin parameters showed excellent reliability, whereas sodium level demonstrated good reliability. The less-reliable grading in sodium could be attributed to its broad reference range as compared to other serum parameters.

### Limitations

The laboratory tests chosen were the most frequently requested indicators of systemic disorders for which differences may exist between the results for blood obtained by 3 mL and 5 mL discarded volume methods. Other blood parameters were not included and may differ. The findings may not apply to different types, sizes, and lumens of CVAD.

### Conclusions

This study concluded that serum parameters obtained with 3 mL and 5 mL discarded volume methods from CVADs have no significant difference. Therefore, the practice of 3 mL discarded volume during blood sampling from CVADs is applicable, instead of the common practice of discarding 5 mL, to reduce blood loss from patients receiving critical care.

### Table 4 Intraclass correlation for reliability of serum parameters at three time points (N = 13)

| Parameter       | Within 3 mL Interpretation | Within 5 mL Interpretation |
|-----------------|-----------------------------|-----------------------------|
| Sodium          | 0.796 Good                  | 0.759 Good                  |
| Potassium       | 0.986 Excellent             | 0.971 Excellent             |
| Calcium         | 0.934 Excellent             | 0.943 Excellent             |
| Creatinine      | 0.996 Excellent             | 0.992 Excellent             |
| Total bilirubin | 1 Excellent                 | 1 Excellent                 |
| Direct bilirubin| 1 Excellent                 | 1 Excellent                 |

Abbreviation: ICC, intraclass coefficient.

- ICC < 0.5: poor reliability.
- ICC 0.5–0.75: moderate reliability.
- ICC 0.75–0.9: good reliability.
- ICC > 0.9: excellent reliability.

which indicates that the difference between the 3 mL and 5 mL discarded volume methods is decreasing at higher values. This is found statistically significant and not clinically important.

The percentage of samples outside CAI for sodium, potassium, and direct bilirubin is 6.25% (95% CI 2.46–15%), and for creatinine it is 7.81% (95% CI 3.38–17%), which is considered small and clinically insignificant as the deviation is found in less than 10% of the samples. Overall, the 3 mL and 5 mL discarded volume methods are considered to be in agreement. This is in contrast to the findings of Cole et al, where calcium levels from the 5 mL discarded volume samples were significantly different than the 2 mL or 3 mL discarded volume samples. However, it was not considered clinically significant in that study.

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Implications

It is essential to sensitize health care professionals about the right blood sampling method and CVAD handling technique. Policies can be developed such that inappropriate blood sampling techniques and high iatrogenic blood loss can be added as indicators for quality assurance. Thus, health care professionals obtaining blood samples from CVADs can contribute significantly in preventing diagnostic blood loss and improving the outcome for critical patients.

Highlights

- There is no clear consensus on discarded volume, as different studies give different recommendations. Blood values obtained using 3 mL and 5 mL discarded volume show clinically negligible difference.
- The 3 mL discarded volume method can be used for blood sample collection from central lines instead of the general practice of discarding 5 mL.
- Inappropriate blood sampling techniques and high iatrogenic blood loss can be added as indicators for quality assurance.

Author Contributions Statement

All the authors listed have made substantial contribution to the conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the design, and final approval of the submitted manuscript version.

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Conflicts of Interest

The authors declare that they have no known competing financial interest or personal relationships that could have appeared to influence the work reported in this paper.

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