Abstract

Objectives: Phlebotomy is presumably the most challenging preanalytical aspect in laboratory medicine. In Europe, inpatient phlebotomy is performed by nurses in 45–60 % of cases. We aimed to develop and test a novel phlebotomy assessment tool for nurses.

Methods: A group of 24 nurses working in a surgical ward was investigated. A three-pronged approach was devised: (1) a standardized knowledge test, (2) three blinded phlebotomy audits, and (3) prospective monitoring of samples sent from the investigated surgical ward for the calculation of preanalytical quality indicators.

Results: The average knowledge test score was 22.7/31 points (12–31, interquartile range 20.5–25). The average audit score was 14.5/18 points (13.7–14.7, interquartile range 14–15). No statistically significant correlations were found between phlebotomy knowledge (or lack of) and corresponding phlebotomy practices (or errors, respectively). Moreover, there was no statistically significant correlation between individual knowledge scores and audit scores. Several misconceptions about the preanalytical phase were identified, along with common phlebotomy errors.

Conclusions: Sometimes, nurses do not follow guidelines due to lack of theoretical knowledge. Other times, nurses fail to follow procedures despite having the prerequisite theoretical knowledge. We observed a discordance between theory and practice regarding certain aspects of phlebotomy. The novel multimodal methodology presented here describes an improved assessment tool and a superior alternative to the popular survey studies. This tool may be used to identify specific and recurrent phlebotomy issues and to improve institutional continuing education programs for nurses through targeted training programs.

Keywords: audit; knowledge test; nurse; phlebotomy; preanalytical phase; quality improvement

Introduction

Phlebotomy is a medical procedure in which a needle is used to draw blood from a vein for blood testing. Phlebotomy can be performed by physicians, specialized personnel (e.g. phlebotomists), nurses, or laboratory technicians. As improper blood drawing techniques may impact laboratory test results, phlebotomy is reportedly the most important preanalytical aspect in laboratory medicine.

According to a survey of 28 European countries, inpatient phlebotomy is performed by nurses in 45–60 % of cases [1]. However, the same survey revealed that specific venous blood sampling training for nurses is offered by the employing institution and has an average duration of only 5 h. This practice is detrimental, especially since nurses trained in continuing education programs were shown to perform better in sample quality than untrained physicians [2]. A variety of guidelines for correct blood sampling are made available by international institutions and national societies: Clinical and Laboratory Standards Institute (CLSI), European Federation of Clinical Chemistry and Laboratory Medicine (EFLM), and World Health Organization (WHO), to name a few [3–5]. In Europe, a working group for the preanalytical phase (WG-PRE) has been created by the EFLM to aid the implementation of correct blood sampling procedures. A 2018 EFLM-COLABIOCLI (Confederación Latinoamericana de Bioquímica Clínica) joint recommendation has been issued and various materials are freely available on the EFLM website: posters, videos, knowledge tests, and guidelines translated into several languages [5, 6]. For a successful implementation of these guidelines, phlebotomy auditing and the use of quality indicators are advised [5]. Knowledge of nurses on phlebotomy and issues that may arise from incorrect blood sampling can be evaluated with the EFLM knowledge test available online [6]. Other knowledge tests have been applied in countries that follow CLSI guidelines or other recommendations [7–9]. Also, a variety of quality indicators are recommended for monitoring the preanalytical phase [10].
Here, we aimed to develop and test a multimodal approach for the assessment of phlebotomy performance in nurses.

**Materials and methods**

In order to assess blood sampling knowledge, practices, and outcomes in the studied group of nurses, a three-pronged approach was devised: (1) a standardized knowledge test as theoretical examination, (2) three blinded blood sampling audits per nurse as practical examination, and (3) monitoring of preanalytical phase QIs as an objective measure of outcomes derived from current phlebotomy knowledge and practices of the investigated group.

The joint EFLM-COLABIOCLI knowledge test [6] (Supplementary Table 1) was translated into the native language of the 24 nurses and applied to all nurses from the surgical ward (n=24). Nurses were not told that the multiple-choice questions had only one correct answer and they were awarded one point for each correctly answered question and zero points otherwise. Total scores were calculated individually for each nurse, as well as for each test item (% of nurses who answered correctly). For comparison purposes, a retranslated version of the knowledge test (from the Romanian translation back to English) can be found in Supplementary.

The audit took place after the knowledge test. The audit form was based on phlebotomy recommendations provided by the EFLM WG-PRE [5]. Each of the 24 nurses was unknowingly audited on three separate blood collections. The single auditor was not part of the 24 investigated nurses. Nurses were blinded to the audit and the auditor was blinded to the results of the knowledge test.

Based on Plebani’s model [10], QIs were calculated for samples sent from the investigated surgical ward to the hospital’s laboratory over a 3-month period: QI-7 (number of test requests with errors concerning test input), QI-10 (number of samples hemolyzed, %), QI-11 (number of sample clotted, %), QI-12 (number of samples with insufficient volume, %), QI-13 (number of samples with inadequate sample-anticoagulant ratio, %), and QI-15 (number of improperly labeled samples, %). The laboratory information system was used to retrieve all data since QIs are routinely monitored and QI statistics are stored in the system.

Statistical processing and analysis were performed in MedCalc® Statistical Software version 20.104 (MedCalc Software Ltd, Ostend, Belgium) and the significance threshold was set at p<0.05. The normality of data sets was tested using the D’Agostino-Pearson statistical test. Based on the statistical distribution of data sets, correlations were tested using either Pearson’s or Spearman’s correlation coefficients. This study has been approved by the local institutional Ethics Committee (approval no. 5729/04.03.2022).

**Results**

**Education level and nursing experience**

Most of the investigated nurses (20/24, 83.3 %) had a post-secondary Nursing degree and between 3 and 39 years of experience (mean 21.1, interquartile range 16–26.5). The other nurses (4/24, 16.7 %) had a Bachelor’s degree in Nursing and one to five years of experience (mean 3.5). The auditor was a fellow Master’s student in Nursing with a previous Bachelor’s degree in Nursing and two years of experience in the investigated surgical ward.

**Phlebotomy knowledge test**

The joint EFLM-COLABIOCLI knowledge test [6] with marked correct answers is shown in Supplementary Table 1, along with the rate of correct answers recorded in the investigated group of nurses. Overall, the knowledge test scores (KS) followed a normal statistical distribution (D’Agostino-Pearson, p=0.09) with an average KS of 22.7 out of 31 points (lowest 12, highest 31, interquartile range 20.5–25). Here, we briefly present the major findings of the knowledge test. In Q1, concerning the percentage of errors that occurred in the preanalytical phase, 19 nurses (79.2 %) chose either 20 % or 40 % instead of 60 %, which is the correct answer. In Q2, regarding the preanalytical steps, nine nurses (37.5 %) did not know that only a minor proportion of preanalytical errors were currently identified by the laboratory, six of them (25 %) believing that doctors can easily detect preanalytical errors. In Q7, 14 nurses (58.3 %) either under- or overestimated how many identifiers are needed for a correct identification of the patient and their sample. In Q8, eight nurses (33.3 %) failed to identify how to correctly confirm the patient’s name. In Q13, 14 nurses (58.3 %) grossly underestimated how soon after an intravenous lipid infusion venous blood should be collected. In Q15, 11 nurses (45.8 %) did not know how serum iron concentration fluctuates during the day, six of them believing that it is constant. In Q17, most nurses identified the correct answer, but 10 of them (41.6 %) added that fist clenching increases the risk of bruising. In Q18, 13 nurses (54.2 %) failed to identify when collection tubes should be labeled. In Q19, 5 nurses (20.8 %) did not know when samples should be mixed, all of them believing that only coagulation tubes needed mixing directly after collection. In Q29, 15 nurses (62.5 %) did not know that even moderate exercise can affect laboratory parameters, most of them answering that only some rare parameters are affected or that only heavy exercise should be avoided. In Q30, 13 nurses (54.2 %) did not know how the body posture of the patient affects laboratory parameters, nine of them believing that the patient always has to lie down during phlebotomy. In Q31, 8 nurses (33.3 %) did not know that even slight underfilling of a coagulation tube can affect coagulation parameters, five of them believing that parameters are affected only if the tube is severely underfilled.
nurse based on the three separate audit results. In the investigated group, APSs followed a normal statistical distribution (D’Agostino-Pearson, p=0.61) with a mean APS of 14.5 out of 18 points (lowest 13.7, highest 14.7, interquartile range 14–15). Due to the large amount of data from the phlebotomy audit, results are graphically presented in Figure 1 (see together with Table 1). During statistical analysis, some questions from the knowledge test were paired with corresponding practical aspects from the audit form (e.g. knowledge about proper patient identification vs. how patients were actually identified in practice). However, in the investigated group of nurses we found no statistically significant correlations between phlebotomy knowledge (or lack of) and paired phlebotomy practices (or errors, respectively). Moreover, there was no statistically significant correlation between KSs and APSs. These results will be further discussed in the Discussion section.

**Quality indicator monitoring**

Over a two-month period, a total of 1,514 chemistry, hematology, and coagulation blood tubes were received by the laboratory from the investigated surgical ward. Some test requests were generated in the laboratory information system, but were either unfinalized or canceled (QI-7, see Table 2). The calculated preanalytical QIs for these samples are presented in Table 2.

The phlebotomy audit form was created based on phlebotomy recommendations provided by the European Federation of Clinical Chemistry and Laboratory Medicine working group for the preanalytical phase (EFLM WG-PRE, 2018). Each of the 24 nurses was unknowingly audited on 3 occasions, resulting in a total of 72 recorded observations for each audit item. The overall rates of success in meeting audit criteria are presented in this table (Overall score – maximum 72).

**Table 1:** Phlebotomy audit and recorded performance.

<table>
<thead>
<tr>
<th>Phlebotomy audit checklist item</th>
<th>Overall score</th>
</tr>
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<tbody>
<tr>
<td>1. Proper patient identification</td>
<td>65 (90.3 %)</td>
</tr>
<tr>
<td>2. Verify patient is fasting and properly prepared</td>
<td>41 (56.9 %)</td>
</tr>
<tr>
<td>3. Obtain all supplies for venous blood collection</td>
<td>70 (97.2 %)</td>
</tr>
<tr>
<td>4. Tube labelling in the presence of the patient</td>
<td>47 (65.3 %)</td>
</tr>
<tr>
<td>5. Put on a new pair of gloves</td>
<td>60 (83.3 %)</td>
</tr>
<tr>
<td>6. Correctly apply the tourniquet</td>
<td>64 (88.9 %)</td>
</tr>
<tr>
<td>7. Proper selection of sampling site</td>
<td>66 (91.2 %)</td>
</tr>
<tr>
<td>8. Proper sampling site cleansing (no subsequent palpation)</td>
<td>67 (93.1 %)</td>
</tr>
<tr>
<td>9. Timely release of the tourniquet</td>
<td>53 (73.6 %)</td>
</tr>
<tr>
<td>10. Gently invert the tubes immediately after collection</td>
<td>71 (98.6 %)</td>
</tr>
<tr>
<td>11. Follow the recommended order of draw</td>
<td>63 (87.5 %)</td>
</tr>
<tr>
<td>12. Remove the needle and activate safety mechanism</td>
<td>67 (93.1 %)</td>
</tr>
<tr>
<td>13. Immediately and safely dispose of the needle</td>
<td>71 (98.6 %)</td>
</tr>
<tr>
<td>14. Properly bandage the puncture site</td>
<td>61 (84.7 %)</td>
</tr>
<tr>
<td>15. Tell the patient to apply pressure and to not bend the arm</td>
<td>72 (100 %)</td>
</tr>
<tr>
<td>16. Invert all tubes at least four more times</td>
<td>28 (38.9 %)</td>
</tr>
<tr>
<td>17. Remove gloves</td>
<td>49 (68.1 %)</td>
</tr>
<tr>
<td>18. Advise the patient to rest for 5 min</td>
<td>33 (45.8 %)</td>
</tr>
</tbody>
</table>

**Figure 1:** Each investigated nurse was given a unique ID between 1 and 24. A single auditor performed 3 separate blinded phlebotomy audits for each of the 24 nurses. The same audit checklist with 18 items (see Table 1) was used on all 72 occasions and results are graphically presented in this figure. Horizontal axis: 24 distinct nurse IDs, each with its own 3 audits. Vertical axis: the 18 checklist items of the audit form. Grey and white areas represent success and failure, respectively, in meeting the audit form requirements. In order to visualize the least and most common blood sampling errors in the investigated group of nurses, audit checklist items have been ranked on the vertical axis from most (top) to least (bottom) fulfilled: 100 % for Q15 and 38.9 % for Q16, respectively.
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Discussions regarding the findings of the knowledge test are based on results briefly presented in the Results section. Extended results are presented in Supplementary Table 1.

Misconceptions about the preanalytical phase

Results from the knowledge test (Q1–Q5) reveal some misconceptions about the preanalytical phase. Most nurses from the investigated group underestimate the frequency of preanalytical errors and 50% believe that preanalytical steps occur during sample preparation in the laboratory. Although they understand that preanalytical errors impact laboratory results, they do not believe that patient safety or outcome can be affected. Moreover, some nurses believe that, even if the laboratory does not detect preanalytical errors, the physicians will easily do that.

Patient identification

Patient identification is the cornerstone of correct laboratory results and it is one of the “Five Rights of Laboratory Testing” [11]. Three out of 31 questions in the EFLM WG-PRE knowledge test (Q6–Q8) address the issue of correct patient identification. The possible answers to question Q8 are 4 questions that may be used to confirm a patient’s identity. In Q8 of the knowledge test, one third of nurses chose to ask a closed question for identifying the patient. A paired error was also recorded during the audits, but to a lesser extent: in 7/72 blood collections, a closed question was asked for patient identification. However, Q1-15 monitoring showed a 0.00% rate of mismatched IDs or improperly labeled samples sent from the audited surgical ward over the two-month period during which the audit was conducted (see Table 2). One possible explanation is that nurses already knew the names of the patients, given the fact that all patients wear an ID bracelet and nurses are assigned to the same rooms. This may explain, but not excuse, the failure to follow the procedure. Another reasonable explanation is that any mismatches that may have occurred between tubes and patient IDs, were not identified by the laboratory. It is reasonable to consider this possibility given that, outside of this study, the real-world rate of mismatched IDs/tubes may be low, but it cannot be zero [12].

Patient preparation

Regarding patient preparation, a high score was obtained in the knowledge test (see Supplementary Table 1, Q9–Q12). However, the phlebotomy audit results showed that in 31/72 blood collections, the patients were not asked if they were in a fasting state. This finding may be partially explained by the investigated surgical ward’s food policy that patients should, at least in theory, not have access to food before early morning blood collections. This is consistent with an Ethiopian study that showed only 63.4% of non-laboratory professionals answered satisfactorily to the questions regarding patient preparation [8]. In a Chinese survey concerning the knowledge of nurses on blood collection, 95% of nurses assessed dietary restrictions, but only 46.6% did the same for strenuous exercise [7]. In our study, only 37% of nurses considered that laboratory results are affected by moderate exercise and 33% believed that only strenuous exercise can impact laboratory results (Q29). Regarding patient positioning, the knowledge test showed similar results: 33% of nurses consider that only rare laboratory tests are impacted by changing the patient’s positioning (Q30). However, we should consider that most patients from the investigated surgical ward are on bed rest and almost all blood collections are performed with the patient lying down.

Regarding how soon blood can be collected after an intravenous glucose or lipid infusion (Q13, Q14), there was a stark difference in knowledge among nurses. While 87.5% answered correctly about the waiting time after glucose infusion, only 41.6% answered correctly about the lipid infusion: a much shorter waiting time was considered necessary, similar to that after glucose infusion. This finding raises important questions concerning the group beliefs and understandings of nurses regarding different intravenous fluids and the metabolism of various nutrients.

<table>
<thead>
<tr>
<th>Quality indicator (QI)</th>
<th>Occurrence rate</th>
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<tbody>
<tr>
<td>QI-7 (no. of test requests with errors concerning test input)</td>
<td>75</td>
</tr>
<tr>
<td>QI-10 (no. of samples haemolysed) – chemistry only</td>
<td>7/501 (1.39%)</td>
</tr>
<tr>
<td>QI-11 (no. of samples clotted) – haematology, coagulation</td>
<td>5/1,013 (0.49%)</td>
</tr>
<tr>
<td>QI-12 (no. of samples with insufficient volume)</td>
<td>2/1,514 (0.13%)</td>
</tr>
<tr>
<td>QI-13 (no. of samples with inadequate sample-anticoagulant ratio) – coagulation only</td>
<td>1/550 (0.18%)</td>
</tr>
<tr>
<td>QI-15 (no. of improperly labelled samples)</td>
<td>0/1,514 (0.00%)</td>
</tr>
</tbody>
</table>

Selected quality indicators (QI) were calculated based on samples sent from the audited surgical ward to the hospital’s laboratory over a 2-month period. *Test requests that were generated in the laboratory information system, but were either unfinalized or cancelled.
Tourniquet time

The effect of prolonged tourniquet time on laboratory results seems to be common knowledge in the investigated group. We report a much higher rate of correct answers (87.5 %) than that reported by the previously mentioned Chinese (18.5 %) and Ethiopian (36.6 %) studies [7, 8]. Contrarily, this finding was not mirrored by the phlebotomy audit results which revealed that, in 19/72 blood collections, the tourniquet was not removed after the blood had begun flowing into the tube. Half of the nurses identified the correct reason why patients should not clench their fists (Q17).

Tube labelling and mixing

When tubes should be labeled is still open to debate: before or after collecting blood? Regardless, guidelines state that tubes should be labeled in the presence of the patient [5]. Regarding this aspect, only 45.8 % of nurses answered correctly in Q18, compared to 90.4 % from the Chinese study [7] and 78.2 % of non-laboratory professionals from the Ethiopian study [8]. Moreover, 25 % of them felt confident that it was correct to label tubes at any time as long as they were sure that the correct labels were used. This finding was also confirmed by the phlebotomy audit results: in 25/72 blood collections, the tubes were not labeled in the presence of the patient.

Regarding tube mixing, the knowledge test states that tubes should be mixed directly after collection and after collection of all tubes (Q19) by inverting at least five times in total (Q20). When asked when and how tubes should be mixed after collecting blood, 79.1 and 87.5 % of nurses, respectively, answered correctly, while 20.9 % considered that only coagulation tubes need mixing directly after blood collection. However, despite this misconception, the phlebotomy audit revealed that, except in one case, all tubes were mixed at least once immediately after collection. The knowledge test showed that nurses understand that tubes should be mixed, but the audit revealed they do not seem to know exactly how: in 44/72 blood collections, the tubes were not mixed additionally after the collection of all the tubes. Thus, the tubes were properly mixed only in 61 % of blood collections, and mixed just once in the other cases. Similarly, the Chinese study reported that 71.9 % of nurses answered that tubes should be inverted twice, and 22.5 % answered that one inversion is sufficient [7]. Insufficient mixing may cause clotting in hematology and coagulation tubes and results from Q28 show that nurses were well aware of this fact. As for QI-11 calculated on the samples received from the surgical ward (Table 2), only 5/1,013 tubes were clotted (0.49 %).

Order of draw, hemolysis, and blood volume

Regarding the order of draw, the scores were high in both the knowledge test (Q21 75 % correct, Q22 83.3 % correct) and the audit (63/72 correct), contrarily to the Chinese study where only 15.5 % of nurses identified the correct order of draw [7]. Most nurses correctly identified the possible causes and consequences of hemolysis. Only 7/501 chemistry samples received from the audited surgical ward were hemolyzed (Table 2), yielding a QI-10 of 1.39 %, comparable or well below the rates of hemolyzed specimens reported by other studies [2, 13–16]. Although only 66.7 % of nurses answered correctly regarding the blood to anticoagulant ratio (Q31), QI-12 was also very low (0.78 %) over the two-month period.

Safety and gloves

All the investigated nurses had good knowledge of how used needles should be handled and what viruses can be transmitted via infected needles. However, the audit revealed that the needlestick safety system was not activated in 5/72 blood collections. In direct observation of the collection technique, a new pair of gloves between consecutive collections was not used in 23/72 audit records, a rate comparable with those reported by other studies involving healthcare professionals [17–19].

Knowledge correlation with practice

A surprising finding of this study was the total lack of statistical correlations between questions from the knowledge test and corresponding practical aspects from the audit records. Thus, more often than failing paired theory and practice together, nurses failed either in theory (meeting the practice) or in practice (knowing the theory). Even when looking at overall individual performance (KSs vs. APSs), there was no correlation between theoretical knowledge and bedside practices. As such, there seems to be an actual discordance between what nurses know and what they do regarding certain aspects of blood sampling. This phenomenon may be rooted in individual and group psychology and remains to be further investigated. Overall, these findings question the validity and real-world relevance of the very popular survey studies where, despite a larger number of participants, nurses are assessed only theoretically through surveys, but no direct bedside observations are performed.
Institutional implications

Institutional continuing education programs for nurses should be adapted to address specific and recurrent issues identified by knowledge tests and direct observations. Implementation of the novel methodology described in this study may improve institutional continuing education programs for nurses by helping in the development of particular corrective measures tailored to focus on specific pre-identified phlebotomy issues.

General considerations, strengths, and limitations

Before concluding, some general considerations are worth mentioning. The hospital policy requires all newly employed nurses to undergo an 8 h initial training. Nurses are then enrolled in continuing education programs that are mandatory on a yearly basis. However, these programs have been suspended in the years 2020 and 2021 as a result of the COVID-19 pandemic. This aspect may have influenced the knowledge and practices of the investigated nurses.

We once again emphasize the importance of standardized tools, such as the EFLM knowledge test and audit form, for assessing health care providers' knowledge and practices, which provide the means for proper quality assessment and laboratory benchmarking. The strength of this study lies in its three-pronged approach: knowledge testing, blinded audits, and targeted monitoring of preanalytical QIs. As such, we consider that the methodology presented in this study describes an improved assessment tool and an objectively superior alternative to the very popular survey studies. Furthermore, the methodology described here enables the development of particular corrective measures such as continuing education programs tailored to focus on specific issues identified during the study. To our knowledge, the present study is the first to apply this multimodal approach for the purpose of identifying phlebotomy-related issues in a group of nurses.

The main limitations of this study are the small number of participants and the fact that all nurses were part of the same ward. However, given the design of this study, it was difficult to ensure blinded audits on a large scale. The surgical ward was chosen specifically to ensure that all participants are blinded in order to avoid any bias occurring from nurses changing their behavior and phlebotomy technique due to the presence of a known observer/auditor (Hawthorne effect).

Conclusions

In this study, we aimed to assess the theoretical knowledge of nurses from a surgical ward regarding venous blood sampling, and how adherent nurses are to blood collection guidelines and protocols. According to our findings, in some cases nurses do not follow the guidelines and protocols due to lack of theoretical knowledge. In other cases nurses fail to follow the procedure despite having the prerequisite theoretical knowledge. There seems to be a discordance between what nurses know and what they do regarding certain aspects of blood sampling. Future studies where this novel methodology would be implemented as an intervention, should be performed on larger cohorts of nurses in order to fully validate this model.

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Research ethics: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the Ethics Committee of the County Emergency Clinical Hospital of Târgu Mureș, Romania (approval no. 5729).

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