Spurious hemolysis, also known as in vitro hemolysis, is the leading cause of unsuitable samples referred for routine and urgent laboratory testing. Among the various sources of spurious erythrocytes injury, collection of blood specimens from intravenous lines (i.e., catheters) is commonplace in short-stay units such as the emergency department (ED), where this practice is virtually unavoidable for a variety of organizational reasons, including faster collection of blood and less discomfort due to additional venipuncture procedures in alternative sites, especially when serial testing is required (e.g., for assessing the kinetics of cardiac biomarkers). Several lines of evidence now attest, however, that this practice is associated with a nearly 6-fold higher risk of hemolysis. The catheter placement site has been proposed as a factor associated with the risk of hemolysis. In a study involving 204 blood specimens collected in an ED, Burns and Yoshikawa found that comparable to that of median anterobrachial vein but lower than cephalic vein (29%; P = 0.01), basilic vein (33%; P < 0.01), and metacarpal plexus veins (75%; P < 0.01). Compared with median basilic and cephalic veins, the relative risk of hemolysis was 1.4 from median anterobrachial vein, 1.6 from cephalic vein, 1.9 from basilic vein, and 4.3 from metacarpal plexus veins.

**Conclusion:** Drawing blood from catheters placed distally from median veins carries higher hemolysis risk.

**Keywords:** preanalytical variability, hemolysis, sample quality, catheter

### Materials and Methods

This investigation was conducted in a large urban ED at the Academic Hospital of Parma, Italy. The study population consisted of all patients admitted during the morning of a single working day who required blood collection for diagnostic purposes. Blood was collected by the 2 nurses on duty the day of the study through a 1.0 mm x 3.2 mm,
20-gauge catheter (Neo DELTA VEN, Viadana, MN, Italy; ref. no. 1331), placed in a vein of the upper limb. Two 13 mm x 100 mm, 5.0 mL Vacutainer® SST II Plus serum tubes (Becton Dickinson Italia S.p.A., Milan, Italy; ref. no. 367955) were collected using a conventional Becton Dickinson (BD) tube holder (BD Vacutainer One Use Holder, Becton Dickinson). The investigation was specifically planned on a single working day to eliminate daily variations in sample collection, handling, and analysis in the central laboratory. The first tube filled was discarded; the second filled tube was rapidly transported to the central laboratory and centrifuged according to manufacturers’ specifications (1300 g for 10 min at room temperature). Serum was tested for hemolysis index (HI) by bichromatic readings at 410/480 and 600/800, along with the other parameters requested by the physician, on a Beckman Coulter DxC chemistry analyzer (Beckman Coulter Inc., Brea CA, USA). Hemolysis was defined as the presence of a value of cell-free hemoglobin greater than 0.5 g/L.1 The statistical analysis, including Wilcoxon-Mann-Whitney test (for continuous variables) and a Chi-squared test (for categorical variables), was performed with Analyse-it for Microsoft Excel (Analyse-it Software Ltd, Leeds, UK). The relative risk (RR) was calculated using MedCalc Version 12.3.0 (MedCalc Software, Mariakerke, Belgium). All ED patients provided an informed consent for participating in this study, which was performed in agreement with the ethical standards established by the institution in which the experiments were performed and the Helsinki Declaration of 1975.

Results

The study population consisted of 67 patients (36 females and 31 males; mean age 56±8 years). Overall, 17 samples were collected from median cephalic vein (25%), 6 from median basilic vein (9%), 16 from median anterobrachial vein (24%), 14 from cephalic vein (21%), 6 from basilic vein (9%), and 8 from veins of metacarpal plexus (12%) (Figure 1). The overall frequency of hemolyzed specimens was 30% (20/67). The mean concentration of cell-free hemoglobin was 0.1 g/L (95% CI, 0.0–0.2 g/L) in samples collected from the 2 median (ie, cephalic and basilic) veins (n = 23; 0.1 g/L; 95% CI, 0.0–0.2 g/L) did not differ significantly from that of samples collected from median anterobrachial (P = 0.11) or cephalic veins (P = 0.22), but...
was significantly lower than that of samples collected from basilic vein ($P = 0.02$) and from veins of metacarpal plexus ($P < 0.01$). Similarly, the frequency of hemolysis in samples collected from median cephalic and basilic veins (4/23, 17%) was comparable to that of samples collected from median anterobrachial vein (4/16, 25%; $P = 0.08$), but was lower than that of samples collected from cephalic vein (4/14, 29%; $P = 0.01$), basilic vein (2/6, 33%; $P < 0.01$) and from veins of metacarpal plexus (6/8, 75%; $P < 0.01$) (Figure 2). As compared with samples collected from the two median cephalic and basilic veins, the RR of obtaining hemolyzed specimens was 1.4 (95% CI, 0.4 to 4.9; $P = 0.56$) in samples collected from median anterobrachial vein, 1.6 (95% CI, 0.5 to 5.5; $P = 0.42$) from cephalic vein, 1.9 (95% CI 0.5 to 8.1; $P = 0.38$) from basilic vein and 4.3 (95% CI, 1.6 to 11.5; $P < 0.01$) from veins of metacarpal plexus.

**Discussion**

Several preanalytical activities, especially those directly related to collection of venous blood, play a substantial role in decreasing the quality of the testing process. Among the various causes of specimen rejection, spurious hemolysis has been reported as the most common reason in studies conducted in several different countries healthcare settings. Blood collection through intravenous lines is commonplace in the ED and other inpatient care settings, due to the efficiency associated with the use of a single venous access for both infusion of therapeutic solutions and necessity for collecting multiple blood specimens. Nevertheless, this practice carries a high risk of erythrocyte disruption, reflected in the high rate of hemolysis. In our study, the rate of hemolysis was 30%. Although the study included a limited number of samples collected from the different veins of the upper arm, we found that the site of catheter placement influenced the rate of hemolysis. Our results suggest that healthcare providers and laboratory professionals should be aware that collecting blood from intravenous lines placed distally from the 2 median basilic and cephalic veins may produce a higher rate of hemolyzed specimens. Furthermore, collecting blood specimens from veins of the metacarpal plexus should be avoided due to the extremely high risk of erythrocyte disruption, which is probably attributable to the large pressure differential that may be exist between small veins, large needles, and evacuated tube systems. Unlike many other studies, this investigation involved blood specimens collected by only 2 nurses within a limited time period, which enabled us to reliably compare the hemolysis rate among specimens collected from different catheter placement sites, eliminating many variables that may be introduced by technique.
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References


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