Clinica Chimica Acta 437 (2014) 187–190

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Interpatient distributions of bloodspot area per fixed volume of application: Comparison between filter paper and non-cellulose dried matrix spotting cards

Laura J. McCloskey, Janet H. Yoo, Douglas F. Stickle *

Jefferson University Hospitals, Philadelphia, PA

ARTICLE INFO

Article history:
Received 18 June 2014
Received in revised form 19 July 2014
Accepted 21 July 2014
Available online 26 July 2014

Keywords:
Bloodspots
Filter paper
Dried matrix spotting cards
Hematocrit

ABSTRACT

Background: Non-cellulose dried matrix spotting (DMS) cards are an alternative to filter paper (FP) for bloodspots. We compared the interpatient distributions of bloodspot areas between DMS and FP for a fixed volume of application of whole blood, and examined correlations of areas with hematocrit.
Methods: EDTA-whole blood adult patient samples ($n = 49$; 25 males, 24 females) were utilized after routine measurement of hemoglobin and hematocrit. Replicate (4×) bloodspots were produced by bolus drop application of 50 μL whole blood via a fixed-volume pipettor to either FP or DMS. Dried bloodspot areas were determined by image analysis.
Results: Hematocrits (HCT) were normally distributed (HCT = 30.9 ± 5.3%). For both FP and DMS, bloodspot areas (a, cm^2) across patients were normally distributed: for FP, $a = 1.11 \pm 0.056$ cm^2 (±5.0%); for DMS, $a = 0.378 \pm 0.037$ cm^2 (±9.9%). Relative bloodspot area differences across the population range were >20% for both DMS and FP. Correlation of bloodspot areas to hematocrit was negative for FP ($r = -0.80$) but positive for DMS ($r = +0.78$).
Conclusions: Interpatient variation in blood volume per area is a preanalytical variable for both DMS and FP bloodspots. Hematocrit is but one interpatient variable, as correlations of fixed-volume bloodspot areas with hematocrit across patients were substantially inexact ($r^2 < 0.65$).

1. Introduction

Filter paper bloodspots have a long history of use in laboratory medicine [1,2]. Applications may usefully be categorized as ranging from qualitative testing (such as in infectious disease screening [3]), semi-quantitative testing (such as in newborn screening [4]), or quantitative testing (such as in therapeutic drug monitoring [5]). There has been a surge of literature in the last few years regarding use of bloodspots [6]. This has been driven in part by interest of the pharmaceutical industry to investigate the potential for use of bloodspots in multiple stages of new drug development and clinical trials [7–10]. When analytical objectives can be met, advantages of bloodspots over blood specimens include ease of transport and storage as well as use of small volumes of blood [6]. Hematocrit is recognized, however, as a variable that can affect bloodspot properties to an extent that can affect certain quantitative analyses [11–14].

Most commonly, bloodspots refer to samples formed on a filter paper (FP) matrix. For FP, numerous studies have shown that hematocrit decreases the bloodspot area per volume of blood applied (e.g., [15]). This may be presumed to be due to effects of hematocrit on rheological properties of whole blood affecting bloodspot formation. In addition to filter paper, however, a non-cellulose matrix is now available (Agilent Bond Elut dried matrix spotting cards (DMS)). Studies have shown the reverse relationship between areas vs. hematocrit for DMS cards: areas increase with hematocrit [14,16].

Hematocrit is unlikely to be the only variable among properties of whole blood affecting bloodspot formation. For example, white cell count, gamma globulins and total protein may reasonably be expected to be interpatient variables that would affect bloodspot formation. Previous studies investigating effects of hematocrit have predominantly used single plasma samples with remix of red cells to control hematocrit as a single variable. It is important also to characterize, more simply, the variation across patients of bloodspot areas for fixed volume of application of whole blood.

2. Methods

EDTA-whole blood adult patient samples were selected randomly from among those submitted to the hospital laboratory for routine measurement of complete blood counts (CBCs). Samples were utilized within 8 h of CBC measurement with interim storage at room temperature. Hemoglobin and hematocrit for each patient sample were recorded as

* Corresponding author at: Department of Pathology, Jefferson University Hospitals, 117 S 11th St, Pavilion 401, Philadelphia, PA 19107. Tel.: +1 215 5035837.
E-mail address: douglas.stickle@jefferson.edu (D.F. Stickle).

http://dx.doi.org/10.1016/j.cca.2014.07.026
0009-8981/

measured by a Sysmex XE-5000 analyzer (Sysmex America, Inc.). After thorough mixing, replicate (4×) bloodspots using either FP (Whatman 903 Sample Collection Cards, GE Healthcare Bio-Sciences Corp.) or DMS cards (Bond Elut DMS Dried Matrix Spotting Cards, Agilent Technologies) were produced by bolus drop application of whole blood via a fixed-volume 50 μL manual pipettor. Bloodspots were dried at room temperature overnight [17].

After drying, 24-bit color, 300 dpi bitmap images of cards were obtained using a desktop scanner (Hewlett-Packard Scanjet G3100). Bloodspot areas were then determined by image analysis (Visual Basic), based on pixel-by-pixel determination of green reflectance to distinguish a red-colored area from a white background. Specifically, average green reflectance (G, 8-bit range of integer values from 0 to 255) within the interior of the bloodspot was used as a center value (C); due to red color of the bloodspot and absorbance of green wavelengths, C was widely separated from (was significantly less than) the maximum reflectance of the non-blood (white) colored area (G > 240). Pixels with maximum reflectance of 1.2 C were counted as part of the bloodspot area. The algorithm was accurate in differentiating blood vs. non-blood areas for both FP and DMS samples.

3. Results

FP and DMS bloodspots were produced from 49 patient samples (25 males, 24 females). Patients ranged in age from 22 to 88 years (median: 57 years; average: 56.4 ± 15.1 years). Hematocrits (HCT) were normally distributed (HCT = 30.9 ± 5.3%; $r^2 = 0.987$) with a range of 19.7–42.7% (Fig. 1).

For both FP and DMS, mean bloodspot areas (a, cm^2) across patients were normally distributed ($r^2 > 0.99$) (Fig. 2). For FP, a = 1.11 ± 0.056 cm^2 (coefficient of variation (CV) = 5.0%), with a range of 0.93–1.25 cm^2. For DMS, a = 0.378 ± 0.037 cm^2 (CV = 9.9%), with a range of 0.30–0.47 cm^2. The ranges of areas across patients for both FP and DMS represented substantial percentage variations. For FP, % differences in areas across the range were +29% (maximum relative to minimum) or −22% (minimum relative to maximum); for DMS, % differences were +60% (maximum relative to minimum) or −38% (minimum relative to maximum). Intra-patient variations among the 4 replicate bloodspot samples for each patient were also considerable for both FP and DMS: average CVs for intra-patient sample areas were 3.06 ± 1.38% for FP, and 5.90 ± 2.72% for DMS.

By linear regression, FP areas were negatively correlated to hematocrit: ($r = -0.80$) (Fig. 3A). In contrast, DMS areas were positively correlated to hematocrit ($r = +0.78$) (Fig. 3B). There was essentially no correlation between patients' FP and DMS bloodspot areas ($r^2 < 0.01$) (data not shown). Patients' DMS bloodspot areas were on average 34 ± 3.2% of their FP bloodspot areas.

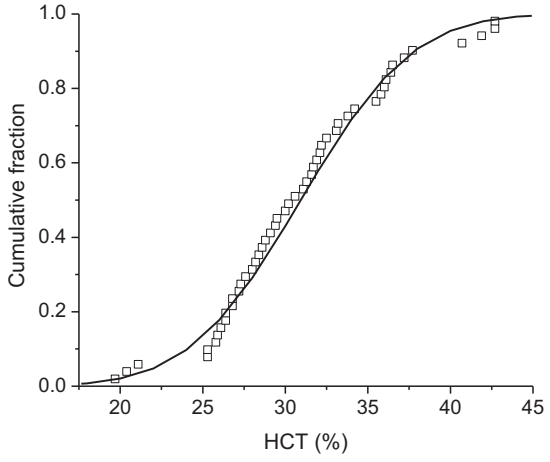

Fig. 1. Distribution of hematocrits: cumulative fraction (y) vs. hematocrit, HCT (x, %). Line: normal distribution: HCT = 30.9 ± 5.3% ($r^2 = 0.987$).

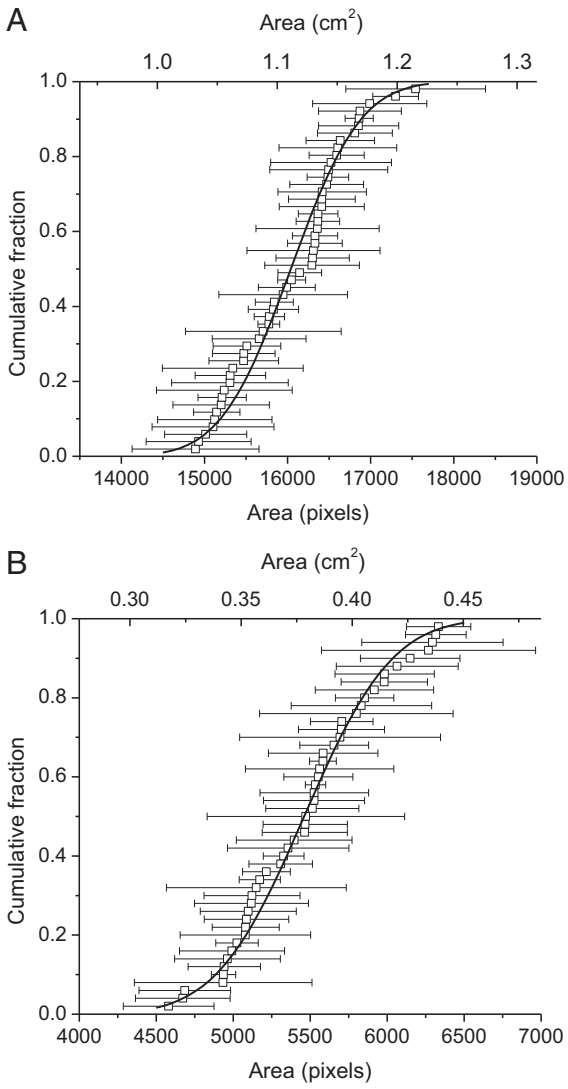

Fig. 2. Distribution of bloodspot areas: cumulative fraction (y) vs. area, a (x, # of pixels; area calibration: 14,432 pixels/cm^2). Points: average ± SD among 4 intra-patient replicate bloodspots. A. FP. Line: normal distribution: a = 16,040 ± 803 pixels ($r^2 > 0.99$) (a = 1.11 ± 0.056 cm^2, range 0.93–1.25 cm^2). B. DMS. Line: normal distribution: a = 5461 ± 542 pixels ($r^2 > 0.99$) (a = 0.378 ± 0.037 cm^2, range 0.295–0.473 cm^2).

4. Discussion

The idealized premise for use of bloodspots is that they provide a vessel of fixed height and infinite width, such that the volume of sample from an interior punch will provide a known volume of homogeneous blood irrespective of the original volume applied [15]. In the idealized case, then, a punch analysis has the capability of providing quantitative analysis without a quantitatively controlled volume of application of sample. The results given here demonstrate departure from the idealized premise according to the interpatient variation in total bloodspot area after a fixed-volume application of whole blood. Interpatient variation of bloodspot areas for fixed-volume manual applications of EDTA whole blood was significant for both FP and DMS, with 5.0% and 9.9% CVs, respectively, and with >20% differences in areas within both of the observed ranges of areas.

Although not measured directly by these experiments, the implication of the variability in areas is that for both FP and DMS there is