
The use of plasma fibrinogen concentrations to predict clinical ischemic events in subjects with and without prior evidence of atherosclerosis [1–14] has led to recommendations to incorporate fibrinogen into the cardiovascular risk profile [15]. Such use of plasma fibrinogen requires careful attention to potential sources of variability, as only small (5%) absolute differences in fibrinogen distinguish subjects who develop cardiovascular disease from those subjects who do not [16].

Variability in fibrinogen results apart from intraindividual differences is difficult to quantify, because the few groups who have examined these issues used different assays for fibrinogen determination. Moreover, the functional assay for fibrinogen is highly influenced by technician expertise and manipulation [17]. Previously, we examined within-run analytical CV, within-person, within-day biological variation, and a 6-week within-person biological variation with the Clauss method for fibrinogen determination [16].

We describe here selected effects of sample collection and storage on fibrinogen results and we propose guidelines for sample collection and storage.

Blood was collected in evacuated tubes (5-mL capacity) containing 0.5 mL of 0.129 mol/L (3.8%) buffered citrate solution (Becton Dickinson). Fibrinogen concentrations were determined by thrombin clottable time with the Clauss method (Dade Data-Fi, Baxter Diagnostics) [18] with bovine thrombin (100 kU/L) and 2.8 × 10−2 mol/L sodium barbital in 0.125 mol/L sodium chloride. Duplicate plasma samples were diluted 1:10 (by vol) with an imidazole buffer (Organon Teknika) and measured at 20- and 40-s intervals at 37 °C in a fibrometer (Model BBL, Becton Dickinson).

All samples were acquired from healthy, nonsmoking volunteers who were seated ≥5 min before phlebotomy [19]. Volunteers were excluded if they had current illnesses, regular and recent use of medications during the preceding 3 months, or an acute-phase illness in the preceding 2 months [20].

The impact of tourniquet pressure and duration was evaluated in a cross-over study design on 38 subjects. The tourniquet pressure was standardized with a sphygmomanometer and a C-clamp that was applied to the tubing to maintain the specified pressure. Mean arterial pressure (MAP) was calculated from three blood pressure recordings obtained at 1-min intervals from seated volunteers. Low pressure was defined as 40 mmHg tourniquet pressure. High pressure was calculated as the MAP + 10 mmHg (MAP + 10) for each subject. Fibrinogen samples were procured at three intervals of equal duration: 0–1 min, 2–3 min, and 4–5 min. For each interval, three replicate samples were acquired at each interval, and the mean value is reported.

On the basis of the findings of the tourniquet pressure and duration study, 14 consecutive blood samples were acquired from each of 38 subjects at low pressure within 1 min. All but the first acquired sample (which was discarded) were centrifuged to obtain plasma specimens. The first four plasma samples were delivered to the Coagulation Laboratory for analysis within 10 min. The remaining nine plasma samples were refrigerated at 4 °C. Three sets of plasma samples were delivered to the Coagulation Laboratory at 6 h, 24 h, and 48 h.

All statistical analyses were performed with SAS statistical package (ver. 6.10) [21], except for the Friedman test performed with SPSS for Windows [22]. Results are expressed as mean ± SD. The first-stick phenomenon was evaluated with a two-tailed paired t-test. The Friedman test was used to examine the influence of the duration of the tourniquet application on fibrinogen concentrations at low and high tourniquet pressures, and the influence of storage time on fibrinogen concentrations. The fibrinogen concentrations at each tourniquet pressure were compared at each time interval by using the paired-samples sign test. A 0.05 significance level was used for statistical tests. A critical difference or relative change value in fibrinogen measurement was defined as 5% on the basis of prospective population studies [16]. The percentage of subjects with a difference in fibrinogen values ≥5% was determined at low tourniquet pressure that was applied for 0–1 min, 2–3 min, and 4–5 min.

The first-stick phenomenon was evaluated in 115 subjects by comparing the fibrinogen concentration from the first-acquired sample (2.58 ± 0.47 g/L) with the average fibrinogen concentration of the next four samples (2.57 ± 0.47 g/L). No statistically significant difference was observed (P = 0.73).

The application of low tourniquet pressure was associated with a progressive increase in the concentration of plasma fibrinogen (P <0.00005) across the three sample acquisition time periods (Table 1). The fibrinogen concentrations were significantly higher for samples acquired during intervals at 2–3 min (2.68 ± 0.70 g/L, P <0.00005) and 4–5 min (2.70 ± 0.71 g/L, P = 0.0005) compared with those acquired during the first minute (2.62 ± 0.63 g/L).
Table 1. Influence of tourniquet pressure on fibrinogen concentration.\textsuperscript{a}

<table>
<thead>
<tr>
<th>Tourniquet pressure</th>
<th>Tourniquet duration, min</th>
<th>P value for trend\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low</td>
<td>0–1</td>
<td>2–3</td>
</tr>
<tr>
<td></td>
<td>2.62 ± 0.63</td>
<td>2.68 ± 0.70</td>
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<tr>
<td>High</td>
<td></td>
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<tr>
<td></td>
<td>2.59 ± 0.42</td>
<td>2.62 ± 0.41</td>
</tr>
<tr>
<td>P value\textsuperscript{c}</td>
<td>0.87</td>
<td>0.13</td>
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</tbody>
</table>

\textsuperscript{a} Fibrinogen concentrations (g/L) are expressed as mean ± SD.

\textsuperscript{b} P value reflects the comparison of fibrinogen values at a particular time interval between low and high pressures with a paired-samples sign test.

\textsuperscript{c} P value reflects the comparison of fibrinogen values obtained at the three time intervals of tourniquet application by the Friedman test.

There was no statistically significant difference between samples obtained at 2–3 min and those acquired at 4–5 min (P = 0.23). Similarly, samples acquired under conditions of high tourniquet pressure increased across the three sampling time periods (P <0.00005). The concentration of fibrinogen was greater for samples acquired at 4–5 min (2.69 ± 0.45 g/L) than those obtained within the first minute after tourniquet application (2.59 ± 0.42 g/L, P <0.00005) or at 2–3 min (2.62 ± 0.41 g/L, P <0.00005). There was no statistically significant difference between samples acquired at 2–3 min and those obtained within the first minute (P = 0.11).

Mean fibrinogen was not influenced by the tourniquet pressure at 0–1 min (P = 0.87), 2–3 min (P = 0.13), or 4–5 min (P = 0.62) (Table 1).

Fibrinogen remained stable at 4 °C for 48 h (P = 0.11) with mean values of 2.53 ± 0.35 g/L at baseline, 2.51 ± 0.36 g/L at 6 h, 2.54 ± 0.40 g/L at 24 h, and 2.57 ± 0.45 g/L at 48 h.

The current recommended procedure for acquiring samples for fibrinogen analysis by the Clauss method mandates that the first sample be discarded [23–25]. Discarding of the first tube of blood or the “first-stick phenomenon”, although not evaluated, has been considered standard practice. This recommendation has been promulgated because tissue thromboplastin could interfere with coagulation assays by activating the intrinsic pathway. For example, the Atherosclerosis Risk in Communities (ARIC) investigators clearly state that the first 3 mL obtained after venipuncture must be discarded [25]. We demonstrate that the first acquired sample can be submitted for fibrinogen analysis.

We have found several published reports that investigate the influence of tourniquet pressure or duration on fibrinogen measurements. The Cardiovascular Health Study investigators reported tourniquet use for only a brief duration in nearly all of the 5000 samples, but 1.2% of phlebotomies required tourniquet application for >2 min [26]. The influence of longer periods of venous occlusion on fibrinogen concentrations was evaluated in several studies. In 25 hyperlipidemic patients, venous occlusion for 10 min resulted in a 3.7% increase in fibrinogen (3.27 to 3.39 g/L, P = 0.052) [27]. Larger increases in fibrinogen after 10 min of tourniquet application were reported for 20 hypertriglyceridemic patients (4.86 to 6.12 g/L or 26%, P <0.0005) and 20 normolipidemic patients (3.42 to 3.96 g/L or 16%, P <0.005) [28]. A 14.5% increase in fibrinogen concentration was reported after 15 min of tourniquet application (3.24 to 3.71 g/L, P <0.001) in 28 healthy subjects [29]. These studies support our results that the duration of venous occlusion has a significant influence on fibrinogen concentrations.

The stability of fibrinogen throughout storage is important for multicenter studies that involve delays from shipping and sample processing. We demonstrate that plasma samples stored at 4 °C remain stable for 48 h. The ARIC investigators examined the impact of temporary storage time on fibrinogen concentration assayed by the Clauss method (1 week at −70 °C vs 30 min before the assay at 4 °C) [26]. They found no significant difference in fibrinogen values with such conditions. Similar findings were obtained in plasma samples stored at 0, 1, 4, and 7 days and assayed fibrinogen by radial immunodiffusion [30].

In this report, we identify tourniquet duration as a critical determinant of preanalytical fibrinogen variability. On the basis of a critical fibrinogen variability of 5%, the application of low tourniquet pressure for 2–3 min resulted in 6 of 38 subjects with differences that exceeded 5%, and this doubled (12 of 38) with 4–5 min of tourniquet application.

We conclude that variability in fibrinogen measurements can be minimized by acquiring samples within 1 min after venipuncture and analyzing samples that have been stored at 4 °C up to 48 h. The magnitude of tourniquet pressure has no influence on fibrinogen concentrations, and the first acquired blood sample can be used for fibrinogen analysis.

This work was supported by an unrestricted research grant from Leo Burnett and Co., Chicago, IL. We acknowledge the contributions of Amy E. McCormick for sample processing, Susan Shott for statistical analyses, and Mary Lou Briglio for manuscript preparation.
References