Intraindividual Variability of Fibrinogen Levels and Cardiovascular Risk Profile

Robert S. Rosenson, Christine C. Tangney, Joanne M. Hafner

Abstract  Prospective population studies have established that fibrinogen is an independent predictor for ischemic heart disease and stroke. These study conclusions have prompted recommendations that fibrinogen determinations be included in the cardiovascular risk profile. The routine availability of fibrinogen measurements may result in widespread screening prior to establishing the validity of a single fibrinogen level as an accurate descriptor for individual subjects. The objectives of this study were to describe the methodological and intraindividual components of variability in fibrinogen measurements determined by using the Clauss method; to establish the usefulness of a single fibrinogen measurement on risk stratification and retest reproducibility; and to determine the influence of intraindividual fibrinogen variability on sample size estimates. Fibrinogen levels were measured by a modification of the Clauss method. Three cohorts of apparently healthy, nonsmoking volunteers were recruited. The single-day intraindividual component of fibrinogen variability was determined in 39 subjects. For the 5-day intraindividual component of fibrinogen variability, 32 subjects were recruited, and in the 6-week intraindividual study, 28 subjects were included. The coefficient of variation for the methodological component of fibrinogen variability was 5.8% as determined from batch analyses, but the intraindividual coefficient of variation for replicate measures on a single day was 10.7%. The 5-day intraindividual coefficient of variation was 14.2%, and for the 6-week period it was 17.8%. Based on the 6-week data, an average of four fibrinogen measures is required to reduce misclassification error to less than 10%. Sample size estimates were made based on predetermined levels of statistical power and the 6-week intraindividual and interindividual variability estimates. The ability to stratify an individual patient based on a single measurement of fibrinogen is limited by methodological and intraindividual components of variability. Clinical studies reporting the influence of specific therapeutic interventions on fibrinogen concentrations must account not only for interindividual variability but for methodological and intraindividual components of variability as well. (Arterioscler Thromb. 1994;14:1928-1932.)

Key Words  • fibrinogen  • Clauss method  • risk stratification

Evidence supporting fibrinogen as a risk factor for cardiovascular diseases derives from population studies, cohort series that demonstrate associations between fibrinogen and disease severity, and the numerous pathophysiological mechanisms by which fibrinogen and fibrin contribute to atherothrombogenesis. Prospective population studies have firmly established that the level of fibrinogen is an independent predictor of ischemic heart disease and stroke with an importance equivalent to blood pressure or cholesterol.

The emerging role of fibrinogen as a predictor of cardiovascular disease and as a marker of atherothrombosis has prompted recommendations that the fibrinogen level be introduced "into the risk profile for cardiovascular disease." The routine availability of fibrinogen measurements may result in widespread screening before the validity of a single fibrinogen determination has been established as an accurate descriptor for individual subjects.

The purpose of this study was to describe the intraindividual components of fibrinogen variability based on replicate measures from a single day, 5 days, and 6 weeks; to establish the usefulness of a single fibrinogen measurement on risk stratification; and to determine sample size estimates considering both the interindividual and intraindividual components of fibrinogen variability.

Methods

Volunteers for this study were recruited from healthy, nonsmoking employees of Rush-Presbyterian-St Luke's Medical Center, Chicago, Ill.

Study Design

The variability in fibrinogen levels was characterized into methodological and intraindividual components. The methodological component of fibrinogen variability was ascertained from replicate measures of pooled blood or batch analysis. The intraindividual components of fibrinogen variability were quantified in individual subjects during three time periods: five consecutive measurements on a single day, single measurements for 5 consecutive days, and nine measurements during 6 weeks.

Blood Collection and Analysis

Venous blood samples were collected between 8 and 10 AM. The participants were not required to submit fasting samples. A tourniquet was applied for the minimal time needed to obtain the replicate samples. The blood was collected from seated volunteers and drawn into 4.5-ML Vacutainer tubes containing 0.5 mL 3.8% sodium citrate. For the consecutive
Intraindividual Variability in Plasma Fibrinogen Measures During Single-Day, 5-Day, and 6-Week Studies

<table>
<thead>
<tr>
<th></th>
<th>Single Day</th>
<th>5 Day</th>
<th>6 Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of subjects</td>
<td>39</td>
<td>32</td>
<td>28</td>
</tr>
<tr>
<td>Mean±SD, g/L</td>
<td>2.58±0.58</td>
<td>2.42±0.50</td>
<td>2.58±0.38</td>
</tr>
<tr>
<td>Median</td>
<td>2.45</td>
<td>2.34</td>
<td>2.51</td>
</tr>
<tr>
<td>Range</td>
<td>0.05-1.85</td>
<td>0-10-2.10</td>
<td>0.50-3.10*</td>
</tr>
<tr>
<td>Coefficient of variation, %</td>
<td>10.7</td>
<td>14.2</td>
<td>17.8</td>
</tr>
</tbody>
</table>

Mean and median are derived from the "ideal" fibrinogen value for all subjects in each study. Range refers to the minimum and maximum difference in fibrinogen values for an individual subject. 
P<.0001 for ranges across the three studies. Six-week ranges were significantly greater (a=0.05) than single-day or 5-day ranges. The coefficient of variation was generated by using a random-effects (type II) ANOVA model.

Results

Components of Fibrinogen Variability

The coefficient of variation for the batch analysis 5.8%, was determined from 480 replicates of pooled blood as performed for routine quality control by Rush Medical Laboratories at Rush-Presbyterian-St Luke's Medical Center. Because tissue thromboplastin has been postulated to interfere with coagulation assays, we performed a substudy that evaluated the influence of the first sample from the Vacutainer with the average of the five subsequent samples from a total of 20 subjects. There was no difference in the concentration of fibrinogen in the first sample compared with consecutively drawn samples (P=.79). Nevertheless, we routinely discarded the first tube for all subsequent fibrinogen analyses.

The cohort for this single-day intraindividual study included 39 subjects (19 men and 20 women) aged 23 to 63 years. The fibrinogen concentration for the 39 subjects averaged 2.58±0.58 g/L (mean±SD) (Table 1). In this cohort, levels of fibrinogen did not increase significantly with age (r=.11, P=.52), and the crude fibrinogen levels were similar in women (2.48 g/L) and men (2.70 g/L) (P=.23). The range in the five consecutive replicate fibrinogen measurements from individual subjects varied from 0.05 to 1.85 g/L (Table 1). The intraindividual coefficient of variation for the 39 subjects having five replicates drawn from the same venipuncture site was 10.7%.

To determine whether samples from subjects with the highest fibrinogen levels had more or less variability in the single-day replicate measures, we determined the correlation between the intraindividual range of fibrinogen levels and the "ideal" fibrinogen level for each subject. There was a significant correlation between range and "ideal" fibrinogen level (r=.34, P=.04).

The cohort for the 5-day study included 32 subjects (15 men and 17 women) whose ages ranged from 21 to 51 years. The range in the five consecutive daily fibrinogen values from individual subjects varied from 0.10 to...
Risk Stratification by Level of Fibrinogen

The ability to categorize an individual subject into broad categories on the basis of fibrinogen levels was calculated by cross-tabulation analysis and logistic regression. Subjects were divided into two groups by using the median of the “ideal” fibrinogen level. The “ideal” level, defined as the average of the maximum number of replicate measures for a given study, clearly has inherent error. Consecutively obtained fibrinogen replicates improved classification accuracy (Table 2). The cross-tabulation analyses showed that a single determination of fibrinogen accurately classifies 87% of subjects compared with the “ideal” fibrinogen value from single-day estimates. Similar classification accuracy was observed with logistic regression analysis (Table 2).

The same approach to classification accuracy was used for the 5-day intraindividual components of fibrinogen variability. A larger misclassification error was observed for the 5-day intraindividual data than was noted with consecutively obtained replicate measures from the single-day study (Table 2). Logistic regression analysis revealed a 19% misclassification error for a single-sample replicate of fibrinogen during the 5-day study. This error was reduced to 10% with two to three replicate measures. The 6-week intraindividual data was associated with the largest misclassification error (Table 2). For a single fibrinogen level, the misclassification error was 32% by cross-tabulation analyses and 21% by logistic regression analysis. The misclassification error decreased to less than 10% with four replicate measurements regardless of the approach used to categorize subjects.

Sample Size Estimates

Sample size estimates were calculated on the basis of 20% and 10% changes in fibrinogen concentrations with an α=0.05 and power (1-β) of 0.80, 0.90, and 0.95 (Table 3). Interventions that would evaluate changes in fibrinogen levels from baseline values are analyzed by using a two-tailed paired t test. For paired t tests, the SD for a single fibrinogen replicate from the 6-week cohort was 0.73 g/L. The average of the first two acquired fibrinogen levels yielded a SD of 0.52 g/L, and the average of three fibrinogen concentrations decreased the SD to 0.42 g/L. For a 20% change in plasma fibrinogen levels with a power equal to 80%, 18 subjects were required when single measurements of fibrinogen are used, and for 90% power, 22 subjects were needed.

Table 3. Power and Sample Size Estimates for 20% and 10% Changes in Plasma Fibrinogen Levels

<table>
<thead>
<tr>
<th>Study</th>
<th>Replicate Numbers</th>
<th>20% Change</th>
<th>10% Change</th>
<th>10% Change</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Power</td>
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<td>2</td>
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</tr>
<tr>
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<td>80%</td>
<td>18</td>
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<td>...</td>
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<td></td>
<td>90%</td>
<td>22</td>
<td>15</td>
<td>...</td>
</tr>
<tr>
<td></td>
<td>95%</td>
<td>27</td>
<td>18</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>80%</td>
<td>37</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>90%</td>
<td>48</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>95%</td>
<td>59</td>
<td>37</td>
<td>34</td>
</tr>
</tbody>
</table>

Ellipsis points indicate a sample size of fewer than 11 subjects.
(Table 3). With two replicate measurements of fibrinogen and a power equal to 80%, only 11 subjects were required. Increasing the number of replicates of fibrinogen reduces the sample SD and hence sample size requirements.

Study protocols that randomly assign subjects to two groups are analyzed with a two-tailed unpaired t-test. For an expected 20% change in plasma fibrinogen levels and on the basis of a single fibrinogen determination, a total of 37 subjects are required for a power of 80% and 48 subjects for a power of 90%. The average of two fibrinogen replicates will markedly reduce the sample requirements to 24 for 80% power and 31 for 90% power.

**Discussion**

The central role of fibrinogen in atherothrombotic disorders depends on its functional activity and protein concentration. The Clauss method is a functional assessment that measures clottable fibrinogen. Of the prospective epidemiological studies, only the Framingham Heart Study and the PROCAM Münster study used functional assays. Whether a functional assay (Clauss, Ratnoff), a quantitative protein assay (immunonephelometric, gravimetric), or the combination provides a better assessment of cardiovascular risk remains undefined.

Variability in levels of fibrinogen determined by the Clauss method results from methodological, intra-individual, and interindividual components. The analytical coefficient of variation or methodological error reported in laboratory manuals is usually determined from batch analysis. The coefficient of variation for the pooled blood in our laboratory is consistent with other centers that report a batch error between 5% and 7% for the Clauss method. The coefficient of variation derived from single-day repeated measurements of fibrinogen was 10.7%, which is nearly double the reported analytical coefficient of variation derived from batch analysis. Therefore, the error associated with single-day replicate samples is probably a more accurate clinical descriptor of fibrinogen variability than the routinely reported batch errors. The ranges in individual fibrinogen values also demonstrate the variability in fibrinogen determinations. For example, one subject had fibrinogen values on the same day that varied by as much as 1.85 g/L.

Factors contributing to the intra-individual and methodological component of fibrinogen variability possibly include duration of tourniquet application, order of sample acquisition, time delay in performing the assay following collection of the blood sample, handling procedures, and technician error. The only factor addressed in the present report was the first "stick" phenomenon. The continued use of the Clauss method for measuring fibrinogen would require careful assessment of these potential sources of error by individual laboratories performing these determinations.

Other assays of fibrinogen concentration reportedly have less methodological variability, but these procedures are more time consuming and less widely available. The gravimetric method for determining fibrinogen has a reported batch coefficient of variation of 1%, and immunonephelometry techniques yield a methodological coefficient of variation between 2.4% and 3.0%.

<table>
<thead>
<tr>
<th>Study</th>
<th>No Detectable IHD</th>
<th>Developed IHD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northwick Park4</td>
<td>2.90 g/L (n=1280)</td>
<td>3.15 g/L (n=109)</td>
</tr>
<tr>
<td>Göteborg²</td>
<td>3.30 g/L (n=608)</td>
<td>3.56 g/L (n=92)</td>
</tr>
<tr>
<td>Leigh³</td>
<td>3.00 g/L (n=209)</td>
<td>3.92 g/L (n=40)</td>
</tr>
<tr>
<td>Münster⁴</td>
<td>2.63 g/L (n=1962)</td>
<td>2.88 g/L (n=82)</td>
</tr>
<tr>
<td>Caerphilly-Speedwell⁷</td>
<td>3.86 g/L (n=4408)</td>
<td>4.09 g/L (n=233)</td>
</tr>
<tr>
<td>Göttingen⁶</td>
<td>3.64 g/L (n=5124)</td>
<td>4.23 g/L (n=107)</td>
</tr>
<tr>
<td>Framingham⁵</td>
<td>≤3.1 g/L (n=911)</td>
<td>&gt;3.1 g/L (n=89)</td>
</tr>
</tbody>
</table>

IHD indicates ischemic heart disease.
not included in this calculation because mean fibrinogen levels for the two groups were not reported.) However, the variability inherent with single determinations of fibrinogen may actually underestimate the strength of the relation between fibrinogen and cardiovascular disease through regression dilution bias.30

For cardiovascular risk factor stratification, accurate classification of fibrinogen levels into upper and lower groups should include four replicate samples acquired during a minimum of several weeks. The routine use of replicate laboratory measures is consistent with the guidelines for lipid levels as espoused by the Adult Treatment Panel of the National Cholesterol Education Program.29

Clinical studies in which the fibrinogen level is reduced either through lifestyle modifications or pharmacotherapy have been based on single measurements of fibrinogen.31,32 These studies are based on small sample sizes, and the authors have often concluded that the same intervention either induced no change in fibrinogen concentration, increased the fibrinogen level, or reduced the fibrinogen level. These conclusions may have resulted from chance alone.

The costs associated with replicate measures of fibrinogen are probably much less than those required for recruitment of a larger sample size. The cost savings are particularly notable when two replicate measurements are made at baseline and follow-up (Table 3).

Vigorous application of the components of fibrinogen variability is essential before recommendations are made regarding inclusion of fibrinogen in the routine biochemical assessment of cardiovascular risk. Most certainly, a single fibrinogen level does not provide confidence in the accuracy of risk stratification. When the Cluss method is used, an average of four fibrinogen measurements is required to reduce misclassification error to less than 10%. The published clinical studies in which interventions are reported to alter the level of fibrinogen must be reevaluated using multiple sampling or larger sample sizes.

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References

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