Fibrinogen: Intra-Individual Variability in Patients with Arterial Disease and in Patients with Cardiac Valve Replacements

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Abstract

Background: Elevated fibrinogen, considered an independent risk factor for coronary disease, stratifies an individual as high risk for coronary disease. A risk marker requires little intra-individual variability during a long period.

Objectives: To establish intra-individual variability of fibrinogen levels in patients with coronary disease.

Methods: We investigated fibrinogen levels prospectively in four blood samples drawn from 267 patients with a history of arterial disease (arterial group) and from 264 patients with cardiac valve replacements (valvular group). The samples were taken during the course of 78.7 and 78.8 days from the arterial and valvular groups respectively.

Results: Marked intra-individual dispersion with a reliability coefficient of 0.541 was found in the arterial group and 0.547 in the valvular group. The Bland-Altman test showed low probability to obtain similar results in different samples from the same individual. These results show large intra-individual variability, with similarities in the arterial as well as in the valvular group.

Conclusions: It is not possible to stratify a patient by a specific fibrinogen dosage.

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In the last few decades several longitudinal epidemiologic studies have identified an elevated plasma fibrinogen level as an important and independent risk factor for developing ischemic heart disease and stroke [1–6]. In order to determine this association it is necessary to assess how precisely an individual is characterized by a single fibrinogen measurement. If the fibrinogen level is to be included in an individual patient's cardiovascular risk profile, it must be reproducible over time within certain limits, and measured not merely over a short period but months apart. When the within-person variability of a biologic measure is relatively high compared to its between-person variability, the association of the result based upon a single measurement with subsequent events may well be underestimated [7,8].

The aim of this study was to evaluate the intra-individual variability of plasma fibrinogen in several samples from two different sets of individuals. In order to analyze a potential different behavior between groups, plasmatic fibrinogen was assayed in patients with arterial disease and in patients who had undergone cardiac valve replacement and had no demonstrable arterial disease.

Patients and Methods

Patients

This prospective study included 531 patients: 267 with a history of arterial disease verified by angiography (the arterial group) and 264 patients who had undergone a prosthetic cardiac valve replacement (the valvular group). Of the 267 patients in the arterial group, 22 had an ischemic cerebrovascular attack, 208 had severe coronary heart disease – which included documented myocardial infarction (n = 32), coronary artery bypass graft (n = 83; 49 patients also had cardiac valve replacement), ischemic dilated cardiomyopathy (n = 66), unstable angina (n = 12), and percutaneous transluminal coronary angioplasty (n = 15) – and 37 patients had obstructive peripheral vascular disease. Patients with a history of repeated respiratory infectious disease, antiphospholipid antibody syndrome, chronic renal failure, or other inflammatory diseases or malignancies were excluded. There were no geographic or genetic differences between groups. Each patient maintained the specific treatment schedule indicated by his or her physician during follow-up. There were no interventions between blood-sampling sessions. All patients received oral anticoagulant therapy with acenocoumarol (Sintrom, Ciba-Geigy) and 91% also took 100–325 mg aspirin daily.

Blood sampling

Blood samples were collected in fasting and resting conditions, between 8:30 a.m. and 10:30 a.m., during the routine follow-up at our center when patients attended for anticoagulant treatment control. On every occasion, before obtaining the blood samples, patients were asked about a possible active inflammatory disease in the previous 2 weeks. Only patients in a steady-state condition were included in the study. Sodium citrate 3.8% was used as anticoagulant (1 volume citrate/9 volumes blood), and centrifuged for 15 minutes at 2,000x*g*. Immediately after, functional fibrinogen was measured by duplicate using Clauss' clotting method with Fibrin-Prest reagent and ST4 instruments from Diagnostica Stago, Asnières, France.

Plasma fibrinogen was measured four times in all patients; the interval between the first and last sample was 78.7 $\pm$ 43.97 days (median 67) for the arterial group and 78.8 $\pm$ 31.1 (median 70) for the valvular group.

Statistical analysis

Continuous data of normally distributed variables were analyzed with the Student's *t*-test for independent samples. An ANOVA model was used when more than two repeated measures occurred (repeated measures ANOVA, Tukey post-hoc test). Categorical variables were analyzed by the Chi-squared test. Pearson's model was carried out to study the univariate

correlation between normally distributed variables, obtaining the corresponding *r* coefficients. Analysis of variance was also used to determine the reliability coefficient *R* [9]. The between-samples agreement was explored using the Bland-Altman technique, considering fibrinogen values as continuous, converting fibrinogen levels in a categorical variable. Kappa coefficient was also obtained. Significance was considered at a *P* level <0.05, two-tailed. Data analysis was performed with CSS Statistica version 5.1/97 for Windows 95.

Results

Table 1 shows that fibrinogen is higher in the arterial than in the valvular group. Median and average values are quite similar, showing a very symmetric distribution. There are no differences between genders (data not shown) in either group.

The inter- and intra-individual variability is presented in Table 2. The reliability coefficient *R* was 0.541 in the arterial group, indicating that results had poor reliability. Similar results were found in the valvular group. Here, the reliability coefficient *R* was 0.547 [Table 2].

With Pearson's correlation coefficient, similar results were obtained for the reliability coefficient *R*, as shown in Table 3. Using the Bland-Altman test, it is possible to precisely extrapolate the values of samples 2, 3 and 4 from the value found in sample 1. Table 3 shows that the possibility of similar results in the fibrinogen level in the subsequent samples is very low because of high value variability in both groups.

Table 1. Fibrinogen level, average ± SD (median) mg/dl, by group

	Arterial group (n=267)	Valvular group (n=264)	*P*
Sample 1	349.5±70.8 (343.2)	319.9±72.7 (316.7)	<0.0001
Range	156–733	130.8–643	
Sample 2	348.3±78.5 (343.2)	327.2±80.4 (325.5)	<0.002
Range	178.3–657.2	124–775.1	
Sample 3	353.2±77.6 (348.2)	333.3±76.1 (330.1)	<0.003
Range	179.1–573.8	156.0–603.4	
Sample 4	369.7±87.7 (356.0)	346.5±82.4 (338.1)	<0.002
Range	184.6–756	142.4–636	

Table 2. Variance analysis in the arterial and valvular groups

Variability	Sum of squares	Degree of freedom	Average of squares
Arterial group			
Inter-individual	4,342,609.9	266	16,325.9
Intra-individual	78,289.9	3	26,096.6
Error	2,277,235.4	798	2,853.7
Total	6,698,135.4	1,067	
F= 9.14, $P < 0.00001$, $R = 0.541$			
Valvular group			
Inter-individual	4,223,165.9	263	16,057.7
Intra-Individual	101,010.7	3	33,670.2
Error	2,175,625.4	789	2,757.4
Total	6,499,802.0	1,055	
F = 12.2, $P < 0.00001$, $R = 0.547$			

Table 3. Pearson's correlation coefficient *R* between samples* and Bland-Altman test

	Pearson's correlation coefficient *R*		
	Sample 1	**Sample 2**	**Sample 3**
Arterial group			
Sample 1	–		
Sample 2	0.554	–	
Sample 3	0.556	0.501	–
Sample 4	0.563	0.491	0.608
Valvular group			
Sample 1	–		
Sample 2	0.574	–	
Sample 3	0.563	0.540	–
Sample 4	0.513	0.492	0.614
	Bland-Altman test		
	Sample 2	**Sample 3**	**Sample 4**
Arterial group	± 137.5 mg/dl	± 136.6 mg/dl	± 143.1 mg/dl
Valvular group	± 138.0 mg/dl	± 135.97 mg/dl	± 148.9 mg/dl

* In all cases $P < 0.001$

Table 4. Kappa concordance coefficient between samples

Fibrinogen (mg/dl)	Kappa (CI 95%)	*P*
Arterial group*		
<300	0.379 (0.330–0.428)	<0.000001
301–399	0.221 (0.172–0.428)	<0.000001
400–449	0.112 (0.063–0.161)	<0.000004
>450	0.292 (0.243–0.291)	<0.000001
Valvular group**		
<300	0.435 (0.386–0.484)	<0.000001
301–399	0.212 (0.163–0.261)	<0.000001
400–449	0.094 (0.045–0.143)	<0.00009
>450	0.225 (0.206–0.304)	<0.000001

* Global kappa coefficient = 0.260 (0.229–0.291), $P < 0.000001$

** Global kappa coefficient = 0.278 (0.245–0.311), $P < 0.000001$

CI = confidence interval

Kappa coefficient also shows that there is no value consistency among samples, notwithstanding the level of plasma fibrinogen obtained in the first sample, as shown in Table 4.

Discussion

Two different aspects have a bearing on the results of fibrinogen dosage: a) the possibility of error in the pre-analytic stage, and b) the variability of results in the same individual (intra-individual variability). Pre-analytic error is not easy to avoid in hemostatic tests. Accuracy in fibrinogen measurements may be improved by using a standardized blood sample collection method and by trying to keep the ratio constant of blood amount/sodium citrate anticoagulant solution volume. Because loss of vacuum or incomplete filling may occur when Vacutainer tubes are used, small changes in the volume of the total blood sample may be responsible for an increased variability in fibrinogen values. Sample collection with a syringe, which allows a stricter volume control, was used in this study as it was

considered a better collection technique than Vacutainer [10]. The equipment for measuring fibrinogen in the pre-analytic stage should be taken into account; we used the ST4 instrumentation recommended by Geffken et al. [11] in their study.

Several drugs, such as ticlopidine, niacin [12] and statins, and coumarin derivatives such as warfarin and acenocoumarin, are known to influence fibrinogen levels. Ticlopidine and fibrates decrease plasma fibrinogen levels [13]. Joukhadar et al. [14] observed that the effect of simvastatin on fibrinogen was more pronounced than the effects of atorvastatin and pravastatin 3 months after statin therapy was initiated. While it is traditionally accepted that oral anticoagulant drugs cause fibrinogen plasma levels to rise, in the study by Lip and co-workers [15] the fibrinogen levels remained unchanged in 10 patients under warfarin treatment during a 2 month follow-up; and in the study by Wallenga et al. [16] fibrinogen levels after 1–3 mg warfarin daily remained unchanged when compared to placebo. Similar findings were reported by Ikuma et al. [17]. These findings were challenged by Vaya and colleagues [18], who observed a small but significant increase in fibrinogen after 1 and 2 months of acenocoumarol therapy compared to the pre-treatment level (332 99 vs. 386 mg/dl, P 0.05).

In order to avoid drug influence on fibrinogen levels in our study, with the exception of small acenocoumarin modification doses, the entire treatment remained unchanged. To analyze intra-individual variability of fibrinogen levels, four consecutive measurements were made in 531 patients – 267 in the arterial and 264 in the valvular group – at intervals of 78.7 $\pm$ 43.97 and 78.8 $\pm$ 31.1 days between the first and the last measurement, respectively. The only difference between groups was a significantly higher level of plasma fibrinogen in the arterial group [Table 1].

The wide range of intra-individual values and the low value of the reliability coefficient R found in both groups reveal that the value of plasma fibrinogen is a poorly reproducible parameter. The R coefficient around 0.54 found in both groups agrees with that obtained by De Bacquer et al. [10] who suggested that a high proportion of the total fibrinogen value variability may be attributed to intra-individual variability, which they estimated in 44% and which reflected an R coefficient of 0.56. A 5 year follow-up study by Meade and associates [19] found that the large variability was mainly the result of an important intra-individual variability (44%). Chambless et al. [20] concluded that fibrinogen is an intermediate repeatability parameter (R = 0.72), but considering that their sample was small (n = 39) and the follow-up less than 30 days, the intra-individual and inter-individual variability may have been underestimated. Marckman and co-workers [21], in a study with a 2.5 year follow-up, estimated that fibrinogen values had poor reproducibility, with R = 0.45.

In epidemiologic studies assessing fibrinogen levels and their relationship with fatal or non-fatal coronary events, the large intra-individual fibrinogen variability would determine a bias in the statistical result assessment. Only R values that are very close to 1 ensure that results will not be influenced by intra-individual variability. Sakkinen et al. [22] found that in 26 healthy individuals four measurements of fibrinogen were required to achieve an intra-individual variation coefficient similar to that of total cholesterol determination, a well-known risk factor for cardiovascular diseases (validity coefficient 0.91).

In conclusion, our data show that plasma fibrinogen level is subject to large intra-individual variations in patients with a history of arterial disease as well as in patients with cardiac valve replacements and no arterial disease. We do not believe that intra-individual variations were the consequence of the drugs' pharmacologic actions because, as previously pointed out, all patients continued their own treatment schedule during follow-up. Taking into account the poor repeatability of fibrinogen, just one fibrinogen dosage higher than normal may be inappropriate for including a patient in a risk group for cardiovascular thrombotic disease. Thus our results suggest that only repeated fibrinogen measurements would indicate whether a patient is in a state of thrombotic risk.

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What is Matter? Never mind
What is Mind? No matter

Punch (1855), now defunct witty British magazine

Capsule

Pepducin-based intervention of thrombin-receptor signaling and systemic platelet activation

Transmembrane signaling through G protein-coupled receptors (GPCRs) controls a diverse array of cellular processes including metabolism, growth, motility, adhesion, neuronal signaling and blood coagulation. The numerous GPCRs and their key roles in both normal physiology and disease have made them the target for more than 50% of all prescribed drugs. GPCR agonists and antagonists act on the extracellular side of the receptors, whereas the intracellular surface has not yet been exploited for development of new therapeutic agents. Covic et al. demonstrate the utility of novel cell-penetrating peptides, termed "pepducins," which act as intracellular inhibitors of signal transference from receptors to G proteins. Attachment of a palmitate lipid to peptides based on the third intracellular loop of protease-activated receptor 1 (PAR1)2 or PAR4 yielded potent inhibitors of thrombin-mediated aggregation of human platelets. Infusion of the anti-PAR4 pepducin into mice extended bleeding time and protected against systemic platelet activation, consistent with the phenotype of PAR4-deficient mice. These investigators show that pepducins might be used to ascertain the physiologic roles of GPCRs and rapidly determine the potential therapeutic value of blockade of a particular signaling pathway.

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Capsule

Making space for cancer therapy

Antitumor immunity can be elicited either by stimulating T lymphocytes *in vivo* by "vaccination" with tumor antigens, or by transferring tumor-specific T cells to patients. Limitations in generating favorable antitumor responses have been encountered in both settings, either because of a failure to overcome prevailing immune tolerance to the tumor antigens or because of poor engraftment of adoptively transferred lymphocytes. Dudley et al. have now combined non-myeloablative conditioning with the transfer of expanded autologous tumor-reactive T cells to treat patients with metastatic melanoma that had proven otherwise poorly responsive to conventional treatment regimens. Several recipients displayed clinical signs of antitumor response and two showed highly significant cancer regression, which correlated with the persistence of lymphocytes with strong tumor reactivity. Although some caution must be exercised in the use of autologous tumor-reactive T cells because of the possibility of associated autoimmune pathologies, these results represent an encouraging development in the use of immunotherapy as a treatment for certain types of cancer.

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