Comparison of an immunochemical assay for plasma fibrinogen and a turbidimetric thrombin clotting technique to discriminate hyperlipidaemic patients from healthy controls

M L Knapp, M D Feher, H Carey, P D Mayne

Abstract
Plasma samples from patients attending
a lipid clinic (n = 14) and healthy control
subjects (n = 21) were assayed for fibrin-
ogen using an immunochemical method
(radial immunodiffusion) and a turbidi-
metric assay based on the thrombin clotting
technique. The patients had sig-
ificantly higher plasma fibrinogen con-
centrations than controls by both meth-
ods, but there was significant overlap
between the two groups when fibrinogen
was assayed by the thrombin clotting
technique; there was almost complete
separation of the two groups using the
immunochemical assay. This difference
in overlap could not be attributed to the
presence or absence of fibrinogen de-
gradation products. These findings may
have important implications for the
choice of method for determining plasma
fibrinogen when assays are used for the
assessment of cardiovascular risk. It is
recommended that plasma fibrinogen
should be assayed by both an immuno-
chemical and a thrombin clotting
method.

Increased plasma fibrinogen concentration has
recently been shown to be an independent risk
factor for the development of cardiovascular
disease.15 In diabetic subjects studies have
shown an association between increased fibrin-
ogen and microvascular and macrovascular
complications.19 Numerous methods can be
used for the estimation of plasma fibrinogen
including thrombin clotting techniques,10-13
heat precipitation,14 salt precipitation15 and
immunochemical assay.16,17

With the aim of establishing a plasma fibrin-
gen method to be used for risk factor analysis
in the routine evaluation of patients we com-
pared the result of fibrinogen assayed by a
thrombin clotting technique and by an immu-
nochemical method (radial immunodiffusion)
in a group of normal healthy controls and
hyperlipidaemic patients.

Methods
All reagents were analytical grade unless other-
wise stated. Thrombin 5000 NIU/phial
was obtained from Rorer Pharmaceuticals,
Eastbourne, Sussex. Protamine sulphate
was obtained from BDH Ltd, Poole, Dorset.
Phosphate buffered saline (pH 6-4) was prepared by dissolving KH₂PO₄
(0·91 g), Na₂HPO₄
(0·47 g), and NaCl (8·5 g) in distilled water to a
final volume of one litre.

Blood was collected from 14 patients attend-
ing the lipid clinic at the Westminster Hospital
(mean age 63 years, range 49-73 years). Five of
the patients were receiving treatment for
hypertension and nine had clinical evidence of
macrovascular disease. All patients were being
treated for hyperlipidaemia (by diet and
drugs). No patient had diabetes; one patient
was a current smoker. Blood was also collected
from 21 healthy normolipidaemic laboratory
volunteers aged 29-40 years. None of the
volunteers was taking any medication and none
had a history of vascular disease. Samples were
collected into sodium citrate, 3 g/l (9 volumes
of blood per volume of anticoagulant), and
centrifuged at 1000 × g for 15 minutes. The
plasma was separated and stored within one
hour of collection at −40°C until assay.
Samples were analysed within one month of

FIBRINOGEN ASSAY
Samples for determination of plasma fibri-
ogen were assayed by an immunochemical
method (radial immunodiffusion) and a turbi-
dimetric method based on the thrombin
clotting technique.13 Samples were thawed at
37°C for one hour before assay. No trace of
fibrin clot was detectable by visual inspection
in any of the samples.

For radial immunodiffusion, commercially
prepared "Partigen" plates were used (Hoe-
chst, Hounslow, Middlesex) Behringwerke
plasma protein standard was used as calibrant
(Behringwerke AG, Marburg, West Ger-
many). The plates were used according to the
manufacturer's instructions. The between
plate coefficient of variation was 5-0% at a
fibrinogen concentration of 4-1 g/l.

Assay of plasma fibrinogen using the turbi-
dimetric method based on the thrombin
clotting technique13 was carried out on the
Centrifichem 600 Centrifugal Analyser (Baker
Instruments, Surrey). Pooled normal human
plasma collected into sodium citrate was used
as standard. The fibrinogen concentration of
this standard (3-6 g/l) was assigned by assay-
ing it five times using radial immunodiffusion
with the Behringwerke plasma protein standard
as calibrant. The clotting reagent contained
thrombin (2250 NIH units/l), CaCl₂ (2·5
mmol/l), and protamine sulphate (10 mg/l)
in phosphate buffered saline, pH 6-4. The throm-
bin was stored at −40°C as a stock solution of
45 000 NIH units/l in 25% (v/v) glycerol and
was added to the clotting reagent just before

Department of
Chemical Pathology,
Westminster Hospital,
Charing Cross
and Westminster Medical
School, London SW1
M L Knapp
H Carey
P D Mayne

Department of
Therapeutics
M D Feher

Correspondence to:
Dr M L Knapp, Department
of Chemical Pathology, St
Peter's Hospital, Chertsey,
Surrey, England

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Comparison of techniques to differentiate hyperlipidaemic patients from healthy controls

Figure 1 Correlation between turbidimetric thrombin clotting technique and radial immunodiffusion for plasma fibrinogen assay. 
- Healthy control subjects; 
- Hyperlipidaemic patients.

Figure 2 Plasma fibrinogen concentrations in healthy control subjects compared with patients. Fibrinogen assay by (a) turbidimetric thrombin clotting technique; (b) radial immunodiffusion. ( ) = samples containing fibrinogen degradation products; ----- median concentration.

use. Briefly, sample (40 µl) and diluent (40 µl H₂O) was mixed with clotting reagent (180 µl) at an analyser temperature setting of 37°C. The increase in absorbance at 340 nm was monitored for between three and 90 seconds and was linearly related to fibrinogen concentration up to at least 7 g/l. The between assay coefficient of variation was 4-2% at a fibrinogen concentration of 3-6 g/l.

ASSAY OF FIBRINOGEN DEGRADATION PRODUCTS

The presence or absence of significant amounts of fibrinogen degradation products in the samples was determined on slides by semi-quantitative latex agglutination immunoassay. ("Fibrinostreum", Organon Teknika Corporation, Durham, USA). This assay is specific for the cross-linked D-dimer domain in fibrin. If cross-linked D-dimer domain fibrin derivatives are detected in plasma, it confirms that thrombin was formed and that factor XIII was activated with reactive in vivo fibrinolysis. The detection limit of this assay is 500 µg/l in fibrinogen concentration equivalents.

Analysis of correlation between the two methods for plasma fibrinogen assay was carried out by least squares linear regression. Deming's correction for slope was not applied because Sex/Sx was < 2.0. The χ² test for 2 x 2 contingency tables incorporating a correction for continuity and the Mann–Whitney U test were used to analyse the significance of any differences between the plasma fibrinogen concentrations observed in the two groups of subjects. The F test was used to analyse the significance of any differences in the variance of plasma fibrinogen values between the groups.

Results

There was a good correlation between the turbidimetric thrombin clotting technique and radial immunodiffusion for plasma fibrinogen assay using the samples from the lipid clinic patients and healthy control subjects (fig 1).

Lipid clinic patients as a group had significantly higher plasma fibrinogen concentrations than the healthy control subjects when plasma fibrinogen was assayed either by the turbidimetric thrombin clotting technique (p < 0.01) or radial immunodiffusion (p < 0.001) (fig 2).

Optimal discrimination of control subjects from hyperlipidaemic patients was achieved at a cut off of plasma fibrinogen of 4-5 g/l for the turbidimetric thrombin clotting technique and 3-5 g/l for radial immunodiffusion. Using these cut off levels, the discrimination of control subjects from patients was significantly better when plasma fibrinogen was assayed by radial immunodiffusion than when measured by the turbidimetric thrombin clotting technique (χ² test: 0.02 < p < 0.05). Furthermore, the variance of plasma fibrinogen concentration observed in the control subjects was significantly less using radial immunodiffusion than by the turbidimetric thrombin clotting technique (F test: p < 0.05), although no significant difference in variance was observed for the lipid clinic patients (F test: p > 0.05).

Only three out of 35 samples were positive for fibrinogen degradation products by latex agglutination immunoassay; one positive sample was from an apparently healthy control subject and the remaining two positive samples were from the patients. All three samples had fibrinogen degradation products at the limit of assay detection (500 µg/l).

Discussion

This study has shown that although there is a significant correlation between the turbidimetric clotting technique and radial immunodiffusion for plasma fibrinogen assay, discrimination between hyperlipidaemic patients and a group of healthy control subjects is better by the immunochemical assay. This may have significant implications for the choice of method for plasma fibrinogen when assays are used for the assessment of cardiovascular risk.

It is unlikely that the improved discrimination by radial immunodiffusion can be attribu-
mented to differences in the amount of in vivo fibrinolysis as only three samples had detectable fibrin D-dimer domain by latex immunodiffusion. It could be argued that the samples from the patients contained more early fibrinogen degradation products (X, Y, and E) which are not detected by the latex immunodiffusion. If present these products would almost certainly cause overestimation of fibrinogen by radial immunodiffusion because of antigenic cross reaction with the intact fibrinogen molecules. Our data do not support this view, however, because the plasma fibrinogen results by radial immunodiffusion are lower in both healthy controls and hyperlipidaemic patients compared with results using the turbidometric thrombin clotting technique.

Discrepancies between plasma fibrinogen assay by radial immunodiffusion and the thrombin clotting technique have been previously described following surgery or extensive intravascular thrombosis and during the menstrual cycle. None of the patients in our study, however, had recently had surgery or extensive intravascular thrombosis. During the menstrual phase, immunoreactive fibrinogen is higher than fibrinogen assayed by the thrombin clotting technique because of the increased concentration of circulating fibrinogen fragments generated by the breakdown of the endometrium.

In our study concentrations of immunoreactive fibrinogen were actually lower than thrombin clottable fibrinogen in both the patient and control groups which makes it unlikely that there were increased amounts of circulating fibrin fragments in our group of patients or controls.

The difference in the median plasma fibrinogen concentrations between the normal healthy controls and hyperlipidaemic patients was similar and was about 1 g/l. It is the lower variance of fibrinogen values seen in the controls when fibrinogen is assayed by radial immunodiffusion that is the major reason for the improved discrimination between the normal controls and hyperlipidaemic patients when using the radial immunodiffusion assay. It is unlikely that a difference in assay precision could be responsible for this as both methods have similar precision.

Our study needs to be extended to ascertain whether similar discrepancies are observed between immunoochemical methods and the clot weight assay and with heat or salt precipitation. Our results may indicate that the significance of plasma fibrinogen concentration as a risk factor for vascular disease may depend on method. Until further work has been carried out, we recommend that for purposes of comparison, plasma fibrinogen should be assayed by both an immunoochemical and thrombin clotting method.

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18. Francis CW, Marder VJ. A molecular model of plasmatic degradation of cross linked fibrin. Semin Thromb Hemostasis 1982;25:35-.
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