In this article

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- This manuscript describes historical aspects and the current status of standardization with respect to measurement of fibrinolytic quantities.

**Abstract**

There is compelling evidence that measurement of analytes of the fibrinolytic system may be helpful for the clinician in a number of situations. Except for measurement of plasma fibrin d-dimer such quantities have not been widely introduced in the clinical laboratory. One main reason for this is a documented large interlaboratory variation for specific analytes. This manuscript describes historical aspects and the current status of standardization with respect to measurement of fibrinolytic quantities. Also, it describes recent initiatives taken within the framework of International Society of Thrombosis and Haemostasis (ISTH) and the International Federation of Clinical Chemistry (IFCC) to promote standardization within the field of fibrinolysis (and coagulation).

**Background**

There has been a great increase in the number of publications dealing with the role of the fibrinolytic system, since thrombolytic treatment of myocardial infarction was introduced in the mid 1980s. Many of such publications have indicated an important role of fibrinolysis in health and disease. Consequently, it has been suggested that measurement of analytes of the fibrinolytic system may be helpful for the clinician in a number of situations (Table 1).

Despite the potential importance of the use of fibrinolytic analytes in a number of patient situations, measurements have only to a limited extent been introduced in the clinical laboratory. One requirement, which must be met, if such analytes should be more widely introduced, is that the results produced in one laboratory are very similar to the results produced in another laboratory. At present, this is hardly the case, and proficiency testing reports have documented a large interlaboratory method-dependent variation in some key variables of the fibrinolytic system (1,2). This significant problem is well-known from other fields within clinical biochemistry. It is the experience that one way to improve the situation is to introduce a reference measurement system (3). However, such a work is complex and comprehensive and it demands cooperation between national and international scientific societies of different disciplines, industry, and official institutions (4).

Here, we briefly summarize the first steps taken in order to try to introduce a standardization system within the field of fibrinolysis.

**The fibrinolytic system**

**Composition**

Four decades ago Astrup proposed that the major role of the fibrinolytic system is to regulate the amount of fibrin deposition in tissue and blood vessels following tissue injury (5,6). Since then comprehensive biochemical studies have elucidated the system in detail. The conversion of M=92,000 g/mol plasminogen to M=70,000 g/mol plasmin is one central step in fibrinolysis. This so-called activation of plasminogen is related to three distinct activator pathways (Fig. 1).

It is believed that the most important activator in blood is plasminogen activator, tissue type, which is a M=60,000 g/mol protein, probably produced and released to blood from the endothelial cells. Plasminogen activator, urokinase type is another M=55,000 g/mol activator,
which can probably be produced and released to blood from endothelial cells in a one-chain pro-enzyme (single chain urokinase plasminogen activator) form and converted to a two-chain form during fibrinolysis. The third pathway is related to contact activation and is yet less well-characterized.

Two serpin class inhibitors contribute significantly to the regulation of activation of blood fibrinolysis. One is plasminogen activator inhibitor 1, which is a 52,000 g/mol plasma protein produced by hepatocytes and endothelial cells. This inhibitor has affinity to both plasminogen activator, tissue type and urokinase type. The primary inhibitor of plasmin is plasmin inhibitor, a M=70,000 g/mol protein produced by hepatocytes.

Fibrin affinity of fibrinolytic components and
the presence of inhibitors secure that the action of plasmin is confined to fibrin. The fibrin affinity is partially regulated by thrombin activable fibrinolysis inhibitor (TAFI), which is a carboxypeptidase that removes COOH-terminal lysine and arginine residues from fibrin (7). The plasmin-mediated degradation of fibrin results in a number of fibrin fragments.

When coagulation factor XIII cross-links the formed fibrin, the degradation products contain fibrin d-dimer, which is a heterogeneous class of degradation products with widely variable molecular weights (average M =182,600 g/mol).

Clinical aspects The haemostatic balance describes the dynamic balance between activation of coagulation and fibrinolysis, which in turn determines the amount of fibrin present following tissue injury. This concept can favourably be used in a clinical setting as depicted in table 2.

Many reports have demonstrated that a defective fibrinolysis characterized by low levels of plasminogen activator, tissue type (enz; procedure), high levels of plasminogen activator, tissue type (imm; procedure), and high levels of plasminogen activator inhibitor 1 (imm; procedure, enz; procedure) is associated with an enhanced risk for evolution of cardiovascular disease. Similarly, it has been reported that determination of plasma fibrin d-dimer is helpful in the diagnosis of exclusion of venous thromboembolic disease. Deficiency of serpin inhibitors (plasminogen activator inhibitor 1, plasmin inhibitor) are associated with an enhanced risk of bleeding (8,9).

Despite the fact that individual analytes of the fibrinolytic system have a prognostic power that equals cholesterol with respect to evolution of future cardiovascular disease, these analytes have not widely been used in daily clinical risk stratification.

Current status of standardization

Most efforts within standardization in fibrinolysis have been associated with determination of the potency of pharmaceutical materials such as recombinant proteins/enzymes used in fibrinolytic treatment. Much less has been done with respect to international harmonization of measurements of fibrinolytic analytes in biological fluids. However, it has been appreciated by different scientific societies that there is a need for increasing the level of standardization, and new activities are now emerging.

Calibration/reference materials

According to ISO a reference material is a material or substance comprising preparations, whose properties are sufficiently homogenous and well-established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials (10). A number of such reference materials exists for measurement of quantities of the fibrinolytic system (11). The values of most materials are assigned in arbitrary units, i.e. international units (Table 3).

These standards have primarily been created by a painstaking work done by Dr. P. Gaffney, The National Institute for Biological Standards and Control, United Kingdom. However, it is apparent from the table that the values of the standards are in arbitrary units (IU). This may be a problem, because the results of a given assay can exclusively be traced back to IU and not to SI units. It is obvious that in complex measurement systems such as e.g. plasma coagulum lysis time (formerly euglobulin clot lysis time) it would be appropriate to express results in arbitrary units, but with respect to well-defined analytes it would be possible to have reference materials with traceability to SI units. Another potential problem with the reference materials listed in table 3 is that the values are usually assigned by collaborative studies, frequently with the use of methods available at time - a procedure which makes it difficult to transfer values from an old to a new standard.

<table>
<thead>
<tr>
<th>Reference material</th>
<th>Unit</th>
<th>Status</th>
<th>NIBSC Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prokallikrein</td>
<td>IU</td>
<td>IS</td>
<td>82/530</td>
</tr>
<tr>
<td>Plasminogen activator</td>
<td>IU</td>
<td>IRP</td>
<td>66/46/urokinase type</td>
</tr>
<tr>
<td>Plasminogen activator</td>
<td>IU</td>
<td>IS</td>
<td>87/594/urokinase type (high molecular weight)</td>
</tr>
<tr>
<td>Plasminogen activator</td>
<td>IU</td>
<td>IS</td>
<td>86/670/tissue type</td>
</tr>
<tr>
<td>Plasminogen</td>
<td>BS</td>
<td>BS</td>
<td>78/646</td>
</tr>
<tr>
<td>Plasmin</td>
<td>IU</td>
<td>IRP</td>
<td>97/536</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor 1</td>
<td>IU</td>
<td>IS</td>
<td>92/654</td>
</tr>
</tbody>
</table>

Table 3

IRP - International Reference Preparation; IS - International Standard; BS - British Standard; NIBSC - National Institute of Biological Standards and Control

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In 1991-1992 meetings between representatives from the Scientific and Standardization Committee (SSC) of the International Society of Thrombosis and Haemostasis (ISTH), the Committee/Commission on Quantities and Units of the International Federation of Clinical Chemistry (IFCC), and the International Union of Pure and Applied Chemistry (IUPAC) resulted in recommendations regarding the use of nomenclature and units within the whole field of haemostasis (12,13). In the foreword of one of the documents it is stated: “The technology used by one laboratory speciality may vary even within the speciality, and may be incomprehensible to another speciality. This is a minor inconvenience to the laboratory specialities, each one essentially operating within its own area of activity. However, for the user this is highly unsatisfactory and also it may hinder the treatment of the patient” (13). Thus, the organisations involved in these standardization activities clearly stress the importance of introducing harmonization in nomenclature and units. Despite this, the recommended nomenclature has so far not been used systematically, neither in daily practice nor in scientific publications. As a first step towards a wide-spread use of a harmonized nomenclature, it may be advised that scientific journals more strictly observe the recommendations.

Methods

In order to secure the highest possible level of accurate results in the clinical laboratory there is a requirement to adhere to the hierarchical structure of a comprehensive coherent measurement system (3,4,14) in which there is a coupling of the analytical method (definitive method, reference method) with reference material (primary reference material, secondary reference material, standards). For quantities for the coagulation system there exists one method adhering to a WHO calibration scheme (plasma coagulation, tissue factor induced (15)), and a number of written national reference measurement procedures. This is the main background that these standardization activities clearly stress the importance of introducing harmonization in nomenclature and units. Despite this, the recommended nomenclature has so far not been used systematically, neither in daily practice nor in scientific publications. As a first step towards a wide-spread use of a harmonized nomenclature, it may be advised that scientific journals more strictly observe the recommendations.
References


