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Review

Anti-Xa bioassays for the laboratory measurement of direct Factor Xa inhibitors in plasma, in selected patients ☆

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ABSTRACT

In the past decade Direct Oral Anti-Coagulants (DOACs), targeting Thrombin or Factor Xa, have enormously facilitated the daily treatment of all relevant patients, including those requiring lifelong therapy. These DOACs have considerable advantages over the use of oral Vitamin K Antagonist (VKA) treatments, in view of having little interferences with food and other medications and also not requiring adjustment for age, gender or weight, with some well-defined exceptions. In this current *What's Happening Section* we focus on measurements of DiXaIs in plasma using anti-Xa assays, with the objective of providing a tribute to Professor Michel Meyer Samama, who was not only a real leader in this field but, in the past, both authors benefited from his wisdom, as a teacher who dedicated his scientific and professional life (among many other interests in hemostasis, thrombosis and fibrinolysis) to develop and promote methods and strategies for laboratory monitoring of anticoagulants. This review presents the performance characteristics of the Anti-Factor Xa assays (measuring Factor Xa inhibition by drugs), which are available for measuring Direct Factor Xa Inhibitors in plasma, and show good compliance of the results with the reference LC:MS method (which measures the mass of Direct Factor Xa Inhibitors). We also present the preparation and validation of drug specific plasma calibrators and controls which are requested for drug measurements. These assays are convenient and practical laboratory tools which can be used in any laboratory setting, and meet the requirements of regulatory bodies for making smart, quantitative, sensitive, accurate and ease of use assays for measuring DOACs when needed. The manuscript focuses mainly on the following areas of current interest: interference in coagulation assays; anti-Xa laboratory methods; development of calibrators and controls for DiXaIs; method validation and comparison with reference techniques (LC:MS); regulatory requirements and method registrations; newer clinical applications and experience on DiXaIs with Anti-Xa assays, and future perspectives.

Contents

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☆ In memory of Professor MM Samama.

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1. Introduction

These past years Direct Oral Anti-Coagulants (DOACs), targeted to Thrombin or to Factor Xa, have been introduced and they greatly facilitate the daily treatment of all relevant patients, many of them on lifelong therapy [1–4]. These DOACs present important advantages over the use of oral Vitamin K Antagonist (VKA) treatments, especially because they have no or little food interaction, little interference with drugs, present a wide therapeutic window, do not need adjustment for age, gender or weight, (except in some extreme circumstances such as a very low weight <40 kg or obesity >120 kg, or when liver or kidney failure is present), and they do not usually need monitoring, except in some specific contexts where the patient is exposed to a high risk of bleeding [5–10]. Adjusted therapeutic protocols and dosages are developed for Asian countries, as Asians tend to have a greater propensity to develop intra-cranial bleeding, especially with VKA treatments, and DOACs reduce that incidence [11,12], but this is still a matter of debate for implementing the best comprise, which ensures the highest antithrombotic efficacy, with the lowest incidence of bleeding risk and side effects.

These DOACs are released for many curative or preventive thrombo-embolic indications, and they are increasingly used for prevention of stroke in patients with nonvalvular atrial fibrillation, which includes a large cohort of patients, which is constantly expanding due to population aging [5,6,8,9,10]. Dabigatran (Pradaxa®, or Prazaxa® in Japan) is the sole Direct Thrombin Inhibitor (DTI) available today [6], whilst 3 different Direct Factor Xa Inhibitors (DiXaIs) are or are being introduced in most countries: Rivaroxaban (Xarelto®), Apixaban (Equalis®) or Edoxaban (Lixiana®) are now available for clinical application [13-18] and others are under current development (Betrixaban).

Although drug monitoring is not usually requested, there are some cases where measurement of DOACs in plasma is useful, and of special help for managing patients at risk. These cases include the control of adherence to a therapeutic protocol, surveillance of drug accumulation risk in long term treated patients (measurement of concentration, just before the next drug intake), overdose, occurrence of thrombotic or bleeding events in treated patients, especially when urgent surgery is needed and the risk of bleeding, which could be induced by a high residual DOAC concentration, must be excluded. Until recently, no specific antidote was available for DOACs [19]. In this context, the presence of a residual high DOAC concentration, scientific societies recommend, when possible, to delay surgery and to undertake it only when this concentration is ≤30 ng/ml especially when the needed surgery is at a high bleeding risk [19-22]. If delay is not possible, a reversal strategy is then necessary [23-26]. Many articles and recommendations have now been published for safely undertaking surgery in DOAC treated patients [27-30].

Measurement of DOACs is also useful for management of patients with mild or more severe impaired renal (especially for Dabigatran and Edoxaban) or hepatic function (all DOACs and more especially for Rivaroxaban and Apixaban), as drug clearance can be reduced and high DOAC concentrations can be present in the circulation [20]. In addition, measurement of the DOAC plasma concentration is also needed in the laboratory setting, in the presence of an unexplained prolonged coagulation time, or for patients admitted in emergency or in intensive care units and for whom use of an anticoagulant therapy is unknown [31]. Global coagulation tests can produce values in the normal range, even in the presence of a significant DOAC concentration which is capable of inducing bleeding episodes. This is of special relevance for Apixaban [32].

The reference method used for measurement of a DOAC concentration in plasma is Liquid Chromatography: Mass Spectrometry (LC:MS). This method was extensively used for validation studies and clinical trials but it cannot be used in emergency conditions or in the usual laboratory setting as it is complex and time consuming [33]. Fast, quantitative, accurate, sensitive, specific and friendly use laboratory assays are then highly expected by clinical laboratories for easy measurement of anticoagulant activity associated with plasma DOAC concentrations when needed [21,23,34]. The general principles of laboratory measurement of Dabigatran concentrations in plasma were recently reported by us in a previous article [35]. In this new report we focus on measurements of DiXaIs in plasma using anti-Xa assays. Expected concentrations can span a dynamic range from <30 ng/ml to >500 ng/ml, and a special focus is required for accurately measuring concentrations at the 30 ng/ml threshold value in patients needing surgery [27,30].

Currently, Anti-Factor Xa kinetic methods are already available for unfractionated or low molecular weight heparins (UFH, LMWH), and they can be adapted for the measurement of DiXaIs, when drug dedicated calibrators and controls are available [35–37]. Specific two-stage assays can also be designed for that application, and they can be made insensitive, in part, to heparins [38,39]. Our objective is to review these assays, their performances and usefulness in clinical laboratory practice.