Thrombosis Research 171 (2018) 14–21

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Full Length Article

Clinical and laboratory diagnosis of autoimmune factor V inhibitors: A single institutional experience

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ARTICLE INFO

Keywords:
Factor V
Thrombin
Hemostasis
Blood coagulation factor inhibitors
Lupus anticoagulant inhibitor

ABSTRACT

Background: Coagulation factor V inhibitors (FV-i) may occur in patients with congenital FV deficiency or previously hemostatically normal patients (autoimmune (AI)-FV-i). Most of the published literature is confined to case reports.

Objective: Describe clinical and laboratory features of AI-FV-i identified through the Special Coagulation Laboratory at Mayo Clinic, Rochester, Minnesota.

Methods: In this retrospective study individuals with FV-i screens performed from January 1999 to February 2017 were identified through the special coagulation laboratory database. Clinical presentation, management, and outcomes were collected for our institutional patients while detailed laboratory data was collected for all tested patients.

Results: Of patients with FV-i managed at our institution, 2/8 (25%) patients experienced no bleeding. There was no correlation between inhibitor titers and/or FV activity (FV:C) levels and clinical bleeding. Hemostatic management included fresh frozen plasma, platelet transfusion, activated prothrombin complex concentrates, and recombinant factor VIIa. Only 2 patients received immunomodulatory treatment. FV-i mixing studies with normal pooled plasma ($n = 26$) demonstrated inhibition on immediate mix but progressive inhibition after 1 h of incubation could not be demonstrated. 71% of platelet neutralization procedures were falsely positive while 59% of DRVVT assays were indeterminate.

Conclusion: FV-i demonstrates immediate inhibition on mixing studies; however our limited data does not support a time dependent inhibition. Our clinical cohort confirms the variable clinical phenotype for individuals with FV-i and supports the notion that management of FV-i should be guided by clinical symptoms and not FV:C or FV-i titer.

1. Introduction

Coagulation factor V (FV) inhibitors are rare and may occur in individuals with congenital FV deficiency or individuals with no previous personal or family history of bleeding (autoimmune FV inhibitors). In the latter group, inhibitors may be idiopathic or associated with surgical procedures, infections, autoimmune conditions, malignancy, and exposure to antibiotics or bovine thrombin [1,2]. The clinical spectrum of presentation of acquired FV inhibitors varies and includes spontaneous life threatening bleeding, post-operative or post traumatic hemorrhage or rarely patients may be asymptomatic and are detected during pursuit of abnormal results of routine tests of coagulation [3]. Laboratory confirmation of FV inhibitors relies on modifications of the Bethesda assay originally described for diagnosis of factor VIII inhibitors.

Management of FV inhibitors depends on clinical presentation. In asymptomatic patients, conservative management includes observation and removal of potential triggering factors such as antibiotics whereas management of symptomatic patients includes achieving and maintaining hemostasis and immune suppression to eradicate the inhibitor. Herein, we review clinical presentation, management, and outcome of patients with FV inhibitors diagnosed and managed at Mayo Clinic, Rochester, Minnesota. In addition, we present our experience with the laboratory testing of FV inhibitors based on our reference laboratory experience.

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https://doi.org/10.1016/j.thromres.2018.09.044
Received 19 June 2018; Received in revised form 22 August 2018; Accepted 4 September 2018
Available online 05 September 2018
0049-3848/

2. Methods

2.1. Study setting, patients and design

In this retrospective study, after institutional review board (IRB) approval, the special coagulation laboratory (SCL) database was searched for individuals undergoing FV inhibitor testing between January 1999 and February 2017. This search identified two groups of individuals: 1) those receiving clinical care at our institution and 2) those undergoing testing as part of our reference laboratory practice (Mayo Medical Laboratories). Details regarding clinical presentation and outcomes were collected only for patients seen at Mayo Clinic, Rochester, Minnesota, while laboratory testing data was collected on both groups.

2.2. Clinical presentation and outcomes for patients managed at Mayo Clinic, Rochester, MN

For patients diagnosed and managed at our institution, outpatient and inpatient medical records were reviewed and data abstracted included patient demographics, clinical presentation, underlying medical comorbidities, medications, management and outcomes. Additional information on blood component administration (e.g. platelets, packed red blood cell transfusions (pRBC), fresh frozen plasma (FFP), intravenous immunoglobulin (IVIG) or cryoprecipitate), and hemostatic agents (e.g. activated prothrombin complex concentrates(aPCC), recombinant factor VIIa (rFVIIa)), and immunosuppressive therapy (glucocorticoids and rituximab) was also recorded. Laboratory data at time of initial presentation, at each hospitalization, and at last evaluation, including results of screening tests and specialized tests of coagulation were collected. Individuals with a history of congenital FV deficiency were excluded from analysis.

2.3. Laboratory assays

The SCL has traditionally offered a comprehensive profile approach to evaluation of coagulation disorders. Components of each profile vary and typically consist of initial screening tests with reflexive mixing studies, coagulation factor assays and inhibitor testing as indicated. Types of profiles include bleeding diathesis profile (for evaluation of bleeding symptoms), prolonged clotting time profile (for evaluation of prolonged PT and/or APTT), lupus anticoagulant, von Willebrand disease and specific coagulation factor inhibitor profiles.

2.3.1. Routine coagulation tests

Reagents and instruments used for testing changed over the study period; all reagents were from USA sources unless stated otherwise. Briefly reagents for PT included Dade Innovin (Siemens, Malvern, PA) and HemosIL™ RecombiPlasTin2G (R2G), (Instrumentation Laboratory, Bedford, MA) and for APTT Platelin (BioMerieux, Cambridge, MA) and HemosIL™ SynthASil (Instrumentation Laboratory, Bedford, MA). Other reagents included thrombin time (TT, HemosIL™ Fibrinogen-C, Instrumentation Laboratory Company, Bedford, MA). Source of normal pooled plasma (NPP), and factor deficient plasma was Precisio*BioLogic*, Inc., (Nova Scotia, Canada). Coagulation factor assays were one stage assays. Over the study period, assays were performed on the MDA-180 (Organon Teknika, Durham, NC), then Sta-R (Stago, Parsipanny, NJ) and more recently ACL TOP 700 (IL, Bedford, MA). All assays were performed according to manufactures' instructions; the lower reportable limit for FV: C was 2%. The change in reagents and instruments over time resulted in shifts of reference ranges, thus, in this publication, it was elected to report clotting time data as prolonged or normal and results of mixing studies as corrected or inhibited, rather than providing reference ranges in the tables. Lupus anticoagulant testing was performed using the platelet neutralization procedure (PNP) (prepared in-house), dilute Russell viper venom time (DRVVT) (CRYOcheck™, LA CHECK™, LA SURE™, Precision*BioLogic*, Inc., Nova Scotia, Canada), and STACLOT-LA (Diagnostica Stago, Parsippany, NJ).

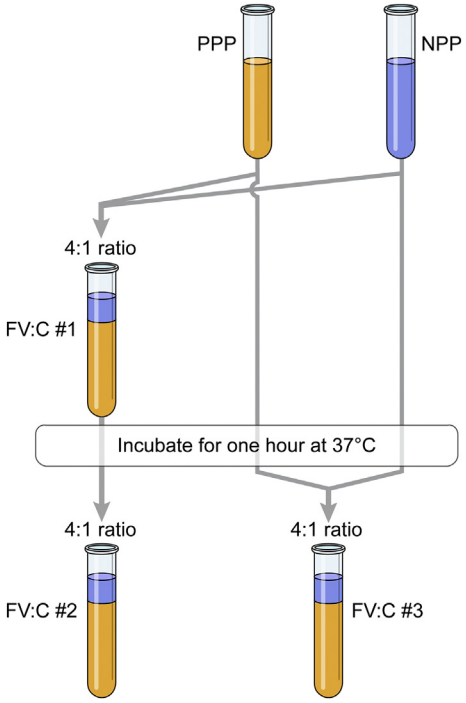

Fig. 1. Factor V inhibitor assay.
Three separate mixing studies are performed with a ratio of 4 parts platelet poor patient plasma (PPP) and 1 part NPP. FV:C #1 is performed immediately after mixing PPP and NPP and FV:C #2 is performed after 1 h incubation of this mixture at 37 °C. Finally, the PPP and NPP are incubated at 37 °C. for 1 h separately and FV:C #3 performed immediately after mixing.

2.3.2. Laboratory characteristics of FV inhibitor testing

Data on results of FV inhibitor testing on samples from both groups were pooled and analyzed. Clinical details on patients managed at our institution were available (one congenital FV deficient patient was excluded from analysis); however, limited clinical information was available on reference laboratory samples. Thus, we were unable to consistently exclude patients who may have had congenital FV deficiency in the latter sample set. For patients with multiple FV inhibitor screens, only the first inhibitor screen results were used for analysis.

2.3.3. FV inhibitor profile

The algorithmic approach to FV inhibitor testing begins with an initial one stage FV activity (FV:C), which if found to be reduced, is followed by a FV inhibitor screening assay and, if positive, is followed by Bethesda titering assay. In the FV inhibitor screening assay, three FV:C results (FV:C #1, FV:C #2, and FV:C #3), obtained from mixing studies performed with a ratio of 4 parts (PPP) and 1 part NPP, are used to determine if the inhibitor screen is positive or negative. All incubations, when performed, are for 1 h at 37° centigrade (C), (Fig. 1). FV:C #1 is performed immediately after mixing PPP and NPP. FV:C #2 is performed after incubation. Finally, the PPP and NPP are incubated separately with FV:C #3 performed immediately after mixing. For ease of description, FV:C #1, FV:C#2 and FV:C #3 are termed mixing study #1, #2 and #3 respectively. The ratio of 4 parts patient to 1 part NPP potentially increases sensitivity of detection of FV inhibitors [4]. The separately incubated samples provide a control for the decay of FV. The FV:C observed after mixing studies are compared to the expected FV:C calculated based on baseline FV:C in PPP and NPP. In general, if the difference between the expected FV: C value and the observed FV: C is