Recurrent thrombosis in patients with antiphospholipid antibodies treated with vitamin K antagonists or rivaroxaban

Ida Martinelli,[1] Maria Abbattista,[1] Paolo Bucciarelli,[1] Armando Tripodi,[1,2] Andrea Artoni,[1] Francesca Gianniello,[1] Cristina Novembrino[1,2,3] and Flora Peyvandi[1,2,3,4]

[1]A. Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico, Milano; [2]Department of Clinical Sciences and Community Health; [3]Laboratory of Clinical Chemistry and Microbiology and [4]Department of Pathophysiology and Transplantation, University of Milan, Italy

Correspondence: ida.martinelli@policlinico.mi.it
doi:10.3324/haematol.2017.185132

Supplemental material for:

RECURRENT THROMBOSIS IN PATIENTS WITH ANTIPHOSPHOLIPID ANTIBODIES TREATED WITH VITAMIN K ANTAGONISTS OR RIVAROXABAN

Ida Martinelli[1], Maria Abbattista[1], Paolo Bucciarelli[1], Armando Tripodi[1-2], Andrea Artoni[1], Francesca Gianniello[1], Cristina Novembrino[1-3] and Flora Peyvandi[1-4]

[1]A.Bianchi Bonomi Hemophilia and Thrombosis Center, Fondazione IRCCS Ca' Granda - Ospedale Maggiore Policlinico; [2]Department of Clinical Sciences and Community Health and [3]Laboratory of Clinical Chemistry and Microbiology, [4]Department of Pathophysiology and Transplantation, University of Milan, Italy

METHODS

Laboratory tests

Venous blood samples were taken at the time of the first visit for thrombophilia testing and collected into vacutainer tubes containing trisodium citrate 109 mM at the 9:1 proportion (blood: anticoagulant). Platelet-poor plasma (PPP) was obtained by centrifugation at 1800g for 15 minutes at room temperature, frozen in liquid nitrogen and stored at -80°C until used.

LA testing was performed on fresh plasma obtained by centrifuging blood samples at 3000g for 15 minutes. The supernatant plasma was re-centrifuged (same conditions) to minimize residual platelets and the samples were analysed within the day of collection. Thrombophilia screening included: DNA analysis (performed on citrated whole blood) for the 1691 guanine to adenine substitution in coagulation factor V gene (factor V Leiden)[1] and for the 20210 guanine to adenine substitution in the 3'-untranslated region of the prothrombin gene;[2] functional and/or antigenic assays for plasma fibrinogen, antithrombin, protein C and protein S;[3] aPL were evaluated as recommended by the Subcommittee on Lupus Anticoagulant/Phospholipid/Dependent Antibodies of the ISTH.[4] LA was detected using two screening tests: silica clotting time (SCT, Werfen, Orangeburg, NY) and diluted Russel viper venom test (dRVVT, Werfen). Mixing tests (patient/normal) and confirmatory tests at increasing phospholipid concentrations carried out whenever the screening test was prolonged beyond the local cut-off values (1.35 ratio for SCT and 1.23 ratio for dRVVT). Patients were considered positive for LA whenever SCT and/or dRVVT gave positive results for screening, mixing and confirm;[5] in patients on VKA therapy mixing studies should correct the prolonged screening studies if they are only due to the anticoagulant effect; the dRVVT with confirmatory testing has been demonstrated to reliably detect LA in patients on warfarin therapy. [6,7] For aCL >40 GPL or MPL and for aβ2-GPI >10 U/mL, corresponding to the 99th percentile, were determined on serum samples by Elisa (Phadia Gmbh, Thermo Fisher Scientific, Uppsala, Sweden) on Phadia 250 analyzer (Thermo Fisher Scientific), following the ISTH recommendation.[4] Patients were considered as having positive results

whenever one of the two tests (aCL or aβ2-GPI) gave results higher than the local cut-off values. The presence of aPL was confirmed in a second blood sample three months apart from the first detection, as recommended by international guidelines.[5] Protein C and protein S were not tested in patients on VKA at the time of blood sampling. Patients on rivaroxaban were tested only for antithrombin and protein S free antigens because of the known influence of the drug on the functional tests; functional protein C was evaluated using a chromogenic assay in order to overcome these interferences.

Statistical analysis

Mean and standard deviation or median and inter-quartile range were used to describe continuous variables. Count and percentage were used for demographic and clinical variables. The incidence rates (IR) of recurrent thrombosis were calculated with their 95% confidence intervals (CI) according to Poisson distribution and expressed as events per 100 patients-year (pts-yr). In case of patients shifted from VKA to DOAC or vice versa, their exposure period was concurrently partitioned to each of the two anticoagulants. Kaplan-Meier curves were used to plot the cumulative incidence of recurrent thrombosis during the follow-up period. The cumulative incidence of recurrent thrombosis was calculated from life table with its 95% CI with Hosmer-Lemeshow-May method for the periods of VKA intake and according to Poisson distribution for the periods of DOAC intake. Hazard ratio (HR) and its 95% CI was calculated by a Cox proportional hazard regression model as a risk estimate of recurrent thrombosis in patients with aPS treated with DOAC relative to those treated with VKA (reference category). All analyses were performed with the statistical software SPSS (release 23.0, IBM SPSS Statistics for Windows, IBM Corp., Armonk, NY, USA).

References

1. Bertina RM, Koeleman BPC, Koster T, et al. Mutation in blood coagulation factor V associated with resistance to activated protein C. Nature 1994;36964–67.

2. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3'-untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88(10):3698–703.

3. Tripodi A. A review of the clinical and diagnostic utility of laboratory tests for the detection of congenital thrombophilia. Semin Thromb Hemost 2005;31(1):25–32.

4. Devreese KMJ, Pierangeli SS, de Laat B, Tripodi A, Atsumi T, Ortel TL. Testing for Antiphospholipid antibodies with Solid Phase Assays: Guidance from the SSC of the ISTH. J Thromb Haemost 2014;12(5):792–795.

5. Pengo V, Tripodi A, Reber G, et al. Update of the guidelines for lupus anticoagulant detection. J Thromb Haemost 2009;7(10):1737–1740.

6. Tripodi A, Chantarangkul V, Clerici M. Laboratory Diagnosis of Lupus Anticoagulants for Patients on Oral Anticoagulant Treatment Performance of Dilute Russell Viper Venom Test and Silica Clotting Time in. 2002;583–586.

7. Olteanu H, Downes K, Patel J, Praprotnik D, Sarode R. Warfarin does not interfere with lupus anticoagulant detection by dilute Russell's viper venom time. Clin Lab 2009;55(3–4):136–42.