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Transient inherited antithrombin deficiency: a real phenomenon?

Martina E. Daly[1], Peter C. Cooper[2], Kieron P. Hickey[2], Ian Jennings[3], Mike Makris[1,2]

[1]Department of Infection, Immunity and Cardiovascular Disease, University of Sheffield, Sheffield, UK; [2]Sheffield Haemophilia and Thrombosis Centre, Royal Hallamshire Hospital, Sheffield, UK; [3]UK NEQAS Blood Coagulation, Sheffield, UK.

Correspondence to:

Dr Martina E. Daly

Department of Infection, Immunity and Cardiovascular Disease

University of Sheffield Medical School

Beech Hill Road

Sheffield, S10 2RX, UK

Email: m.daly@sheffield.ac.uk; Tel: 00 44 114 2713213; Fax: 00 44 114 2711863

Dear Sirs,

We were interested to read the paper by Navarro-Fernández and colleagues regarding antithrombin Dublin (p.Val30Glu) and its capacity to cause transient antithrombin deficiency (1). Antithrombin Dublin was first described by one of us in 1987 when it was identified as part of a study that aimed to determine the effects of asparaginase therapy on the different plasma isoforms of antithrombin that can be distinguished by isoelectric focusing. The Dublin variant was found to be more negatively charged when compared with normal antithrombin (2). Sequencing of *SERPINC1* encoding antithrombin subsequently revealed a single nucleotide variation predicting a p.Val30Glu substitution in the signal peptide of antithrombin which redirects signal peptidase cleavage to a site two amino acids into the mature protein and results in removal of the N-terminal dipeptide from antithrombin (3). Of note, while none of the heterozygous carriers of the Dublin variant identified in the original study had a history of thrombosis at the time of investigation, in our subsequent study we reported the Dublin variant in a patient with a history of spontaneous deep vein thrombosis and pulmonary embolus at age 41 and the presence of anti-IIa and anti-Xa activity levels at the lower end of the normal range (2,3). Its variable association with thrombosis and the observation that the Dublin variant, denoted rs2227624, is relatively prevalent in the European population (MAF 0.0017 in the ExAC database (http://exac.broadinstitute.org/variant/1-173884010-A-T) have meant that its clinical significance has remained elusive.

Navarro-Fernández et al (1) observed that recombinant antithrombin Dublin adopted a hyperstable conformation of reduced activity when it was expressed in HEK cells. This led them to speculate that the p.Val30Glu mutation facilitates the formation of disulphide-linked polymers of antithrombin, similar to those reported for antithrombins Wibble and Rouen VI, two variants caused by mutations that facilitate the conversion of antithrombin to its latent form and the development of antithrombin deficiency under conformational stress conditions such as pyrexia (4,5). However, they did not identify the factors that might have triggered the conformation stress conditions in the symptomatic carriers of the Dublin variant in their study.

We recently identified antithrombin Dublin in a patient who developed a pulmonary embolism during pregnancy. Her thrombin-based antithrombin levels (Berichrom, Sysmex UK) on the ACL TOP analyser (Werfen UK) were initially reduced (0.72, 0.69 and 0.73IU/mL) but increased and indeed normalised after delivery (1.04, 0.92 and 0.86IU/mL). Antithrombin antigen (ELISA) agreed well with antithrombin activity levels during pregnancy (0.75, 0.76, 0.74IU/mL) and after delivery (0.98IU/mL). Our adult reference ranges for antithrombin activity and antigen are 0.85-1.31IU/mL and 0.83-1.24IU/mL, respectively, and our antithrombin activity inter-assay coefficient of variation (CV) is 2.5% at an antithrombin level of 0.92IU/mL (95% limits 0.87-0.96IU/mL, n=83). The patient did not have an increased body temperature or fever at the time of her thrombotic event, leading us to speculate that the classical triggering factor of pregnancy may have predisposed her to the development of transient antithrombin deficiency that may be associated with the Dublin variant, though other possible explanations for variations in antithrombin level, including pre-analytical variables, cannot be excluded.

Interestingly, we noticed that the patient had specimens with poor anti-Xa duplicate assay levels (Coamatic assay, Quadratech UK, on Sysmex CS-5100 analyser) in response to low molecular weight heparin treatment, in two samples during the pregnancy, duplicates were 0.29 and 0.65IU/mL and in another sample, 0.33 and 0.59IU/mL. These two pregnancy samples were assayed for factor Xa-based antithrombin level (Innovance, Sysmex) and results were borderline at 0.85 and 0.81IU/mL (adult reference range 0.85 – 1.15IU/mL) and higher than the thrombin-based assays (0.72 and 0.69IU/mL). Her samples, when tested repeatedly, yielded a CV of approximately 20% in comparison to a control sample with CV of 10%. The reasons for the unexpected poor precision in the patient's anti-Xa levels, and the higher Xa-based antithrombin are unexplained, but it would be interesting to know if low molecular weight heparins affect antithrombin levels in carriers of the antithrombin Dublin variant, and if so, to what extent.

Whilst our observations above are consistent with the findings of Navarro- Fernández et al (1) we would like to add a word of caution regarding measurement of antithrombin levels. A number of different assays for antithrombin activity are in use by different laboratories, and this can contribute to variation in results. The majority of laboratories will only employ one assay for antithrombin activity, and some defects in the antithrombin gene have been

shown to give markedly different results with different assays (6). In the authors' laboratory, at an antithrombin level of 90 IU/dL, the inter-assay coefficient of variation for bovine-thrombin-based and FXa-based chromogenic antithrombin assays is approximately 3%, and at a level of 50 IU/dL it is approximately 4%. Recent EQA data (UK NEQAS, unpublished) demonstrate generally good agreement between antithrombin assay results in different laboratories, with CVs of between 5 and 8%, and comparable results for different assays using either bovine thrombin- and Xa-based methods for samples with normal antithrombin levels. However, results within individual laboratories can range from 29u/dl to 101u/dl when testing the same sample, and for a sample with reduced levels of antithrombin, a statistically significant difference in results obtained with Xa-based and bovine thrombin-based methods was observed (56u/dl, n=179 and 62.1u/dl, n=124 respectively, $p<0.001$). It is therefore important to consider individual results in the context of this variation. Review of the literature does not reveal a consistent association between transient antithrombin deficiency and a specific activity assay in carriers of antithrombin Dublin. However, the number of cases reported, for whom antithrombin levels are also available, is small, and we cannot rule out an assay-specific effect. Interestingly, Puurunen et al (7) identified the Dublin variant in four members of a Finnish family, two of whom had clear type II deficiency while the other two had normal levels of antithrombin activity and antigen. Measurement of the antithrombin activity results in the two carriers with type II deficiency using five different assays showed concordance in the two carriers with type II deficiency (7). In the study by Navarro- Fernández et al (1), there were likely to be differences in the assays used to diagnose antithrombin deficiency at the hospital of origin, and the central reference laboratory. The results seen in our patient would suggest however, that the differences they observed were genuine and not due to assay variation.

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