TAFI(a) Assays Measure Extent of Activation in Patient Populations

Written by **Rita Buckley**

Assays for the activated form of thrombin activatable fibrinolysis Inhibitor(a) (TAFIa) can measure the extent of TAFI activation in patient populations. TAFI is a human plasma zymogen that is related to pancreatic carboxypeptidase B (CPB). The active form of TAFI (TAFIa), formed by thrombin cleavage of the zymogen, likely inhibits fibrinolysis by removal of the carboxyl-terminal lysine residues of partially degraded fibrin that stimulate plasminogen activation. TAFI is encoded by the CPB2 gene (chromosome 13) [Boffa MB et al. *Biochemistry* 1999]. Ann Gils, PhD, Katholieke Universiteit, Leuven, Leuven, Belgium, presented insights into the function and regulation of TAFI.

During the cloning of TAFI cDNA from several human liver cDNA libraries, Zhao and colleagues [*Thromb Haemost* 1998] identified a single nucleotide polymorphisms (SNP) at position 505A/G in the coding region of the TAFI gene that resulted in a substitution of alanine for threonine at residue 147. Brouwers et al. [*Blood* 2001] identified another SNP, 1040C/T, in the coding region of the TAFI gene by comparing published sequences (GenBank no. NM_001872 and NM_016413). This SNP also resulted in an alanine substitution (Thr325Ile) at residue 325.

It was previously shown that commercially available antigen assays underestimate the TAFI-Ile325 concentration (eg, 44±8.9% and 100±30% for the Ile/Ile and Thr/Thr isoforms, respectively) [Gils A et al. *Arterioscler Thromb Vasc Biol* 2003]. On the other hand, studies using recombinant proteins have demonstrated a functional effect of the Thr325Ile substitution on the stability of activated TAFI resulting in altered antifibrinolytic activity [Brouwers GJ et al. *Blood* 2001].

Quantifying and detecting TAFI, TAFIa, and TAFIai can be accomplished via direct (ELISA or functional assay) or indirect measurement. The latter includes, among others, animal models and the Chandler Loop (Figure 1) [Vercauteren E et al. *J Thromb Haemost* 2013; Mutch NJ et al. *J Thromb Haemost* 2003].

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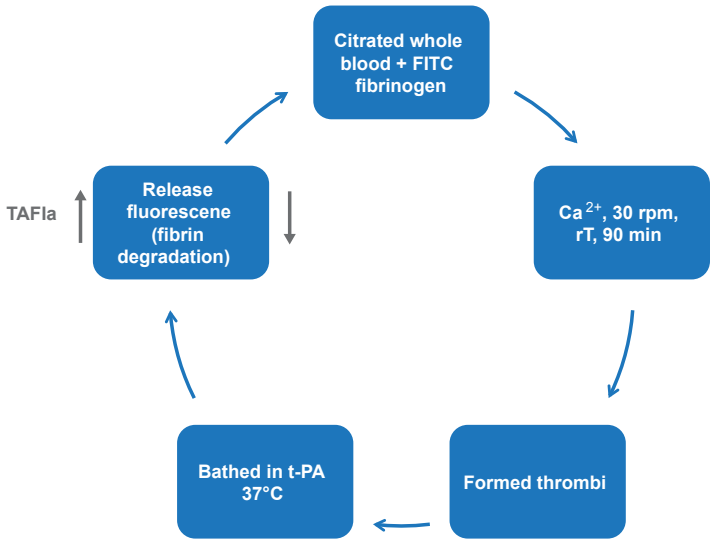
Figure 1. The Chandler Loop System



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According to Prof. Gils, many pharmaceutical companies are attempting to inhibit TAFIa using TAFI-LMW, including 2-mercaptocyclopentanecarboxylic acid WO 10/0330064 and aryl guanidinic TAFIa inhibitors WO 03/080631.

Conclusions from mouse monoclonal antibodies in the pharmacologic inhibition of TAFI-Ab show that activation of TAFI as well as TAFIa can be targeted. T/TM as well as plasmin-mediated TAFI activation seems important. *In vivo* studies are scarce due to lack of cross-reactivity with mouse or rat TAFI.

Pharmacologic inhibition of TAFI-Nb has shown that TAFI and the activation of TAFI and TAFIa can be targeted. Further, due to their size, VHH has better clot penetration. The short half-life and lack of cross-reactivity with mouse and rat TAFI hampers studies in mouse models. TAFI also plays a role in inflammation, with TAFIa inactivating C3a, C5a, thrombin cleaved osteopontin, and bradykinin.

Prof. Gils explained that in time the toolbox of TAFI(a) inhibitors allows to investigate the role of TAFI inhibition using animal models whilst the availability of assays to measure the extent of TAFI activation allows to explore the role of TAFI (activation) in different pathologies.

Phenotyping and Genotyping of Platelet Disorders

Written by Rita Buckley

Investigation of patients with mild bleeding disorders might provide novel information on the regulation and role of platelet proteins. It might even identify new targets for prevention of thrombosis. However, gene mutations require phenotypic support to assign causation, according to findings from the observational study, Genotyping and Platelet Phenotyping [GAPP; ISRCTN77951167; UKCRN ID 9858]. Steve P. Watson, PhD, University of Birmingham, Birmingham, United Kingdom, presented results to date from the study.

Several factors have contributed to the fact that platelet function disorders are heavily underdiagnosed, including the absence of a "gold standard" point-of-care assay of platelet function and the variable penetrance of bleeding in families with inherited disorders of platelet function. The GAPP study is testing the hypothesis that a proportion of patients who present with excessive bleeding have a previously unrecognized impairment in platelet function that may explain their propensity to bleed in conditions that would not normally be associated with severe bleeding.

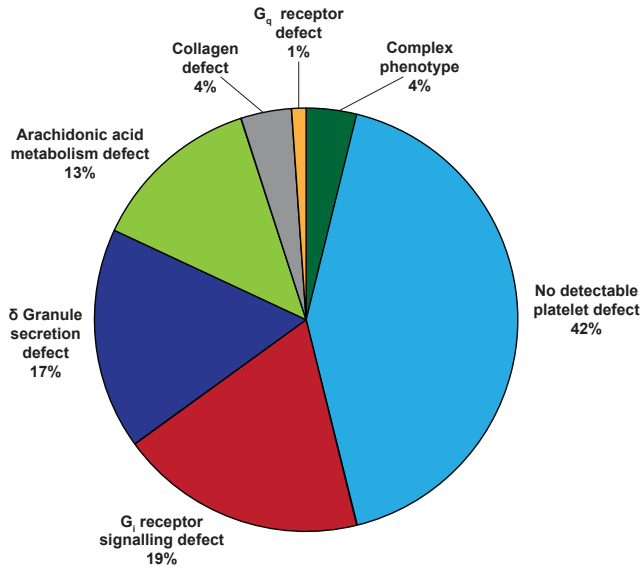
The study uses a combination of platelet phenotyping and a combination of targeted and whole exome gene sequencing to identify candidate mutations underlying platelet dysfunction. The effect of a small number of missense mutations discovered in the study on protein function is being investigated through expression studies in immortalized cell lines

To date more than 520 participants, including patients with excessive bleeding suggestive of inherited platelet dysfunction and healthy volunteers, have been recruited to this multicenter study. The main inclusion criteria for patients are patients of any age with excessive bleeding who are willing to participate and are able to provide informed consent. Exclusions include known platelet disorders, such as Glanzmann's thrombasthenia, Bernard Soulier syndrome, Hermansky Pudlak syndrome, and May Hegglin anomaly.

Today, light transmission aggregometry (LTA) is used worldwide for the study of heritable platelet function disorders (PFDs), but interpretation of results is complicated by the feedback effects of adenosine diphosphate (ADP) and thromboxane A(2) [TxA(2)] and the overlap with the response of healthy volunteers [Dawood BB et al. *Blood* 2012].

The GAPP study performed lumi-aggregometry on 9 platelet agonists in patients with suspected PFD and in healthy volunteers. Abnormal LTA or adenosine triphosphate (ATP) secretion test results were identified in 58% of patients in the GAPP study. In 84% of these, the patterns of response were consistent with defects in Gi receptor signaling, the TxA(2) pathway, and dense granule secretion. Targeted genotyping identified three participants with function-disrupting mutations in the p2Y (12), ADP, and TxA(2) receptors (Figure 1). Prof. Watson noted that the majority of defects were in platelet feedback pathways: ADP, thromboxane, and secretion.

Figure 1. Classification of Defects



The majority of defects are in platelet feedback pathways: ADP, thromboxane and secretion.

Adapted from Dawood BB et al. Evaluation of participants with suspected heritable platelet function disorders including recommendation and validation of a streamlined agonist panel. *Blood* 2012;120:5041–5049.