

Understanding the complexity of abciximab-related thrombocytopenia

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Patients with a rare bleeding disorder characterised by skin and mucosal bleeding – typical of thrombocytopenia – but with a normal platelet count, and later characterised by a defective aggregation to all agonists, described as Glanzmann thrombasthenia, have mutations in a platelet membrane glycoprotein (GP) called GP IIb/IIIa, also known as the integrin α IIb β 3 (1). Such discovery paved the road to a molecular understanding of how platelets aggregate, with GPIIb/IIIa acting as a main ligand for fibrinogen, which in turn provides the main molecular bridge between two adjacent platelets. This was the basis, now more than 15 years ago, for starting the development of a new class of antiplatelet agents directed against GP IIb/IIIa, blocking the final common pathway of platelet aggregation. The first such drug was derived from a murine monoclonal antibody (7E3) recognising activated, but not resting platelets, and binding to an epitope on the GPIIb/IIIa complex close to a critical binding site for fibrinogen (2). To decrease the immunogenicity of the antibody, the pharmaceutical abciximab (ReoPro, developed by Centocor and Eli Lilly), was produced. Abciximab is a chimeric (human/mouse) Fab fragment derived from 7E3, where the N-terminal sequences that control its specificity were incorporated into a human IgG1 framework. The intact chimeric IgG molecule was then cleaved by papain to produce the Fab fragment abciximab. Ab-

ciximab is therefore a Fab chimera that retains the mouse-derived variable portion of murine 7E3 joined to the constant region of human IgG Fab.

Out of several other molecules developed for intravenous and oral use to target GP IIb/IIIa, two other compounds of this class have become available for intravenous use only. One is the Lys-Gly-Asp (KGD)-containing cyclic heptapeptide eptifibatide (Integrilin, developed by Schering-Plough), derived from disintegrins, a class of proteins found in snake venoms and interfering with the binding of Arg-Gly-Asp (RGD)-containing adhesive proteins to cellular integrins. The other is tirofiban (Aggrastat, developed by Merck), developed by engineered synthesis to mimic the charge and spatial conformation of the RGD sequence. These compounds, which are small molecules not per se immunogenic, are collectively termed ligand-mimetic.

Abciximab, eptifibatide and tirofiban are currently approved and recommended for therapeutic use in acute coronary syndromes (both ST-elevation myocardial infarction and non-ST elevation acute coronary syndromes), especially in the setting of percutaneous coronary interventions (PCI) (3, 4). Here, in patients with complex anatomy and receiving coronary stents, in the highly thrombogenic setting of acute coronary syndromes, abciximab is currently, within the category, the agent of choice when given at the time of PCI, having shown superiority in one face-to-face trial against tirofiban (5).

In earlier clinical trials (6, 7) and in immediate post-marketing surveillance of abciximab, it was found that about 1% of patients develop thrombocytopenia. Thrombocytopenia (nadir platelet count $<100 \times 10^9$ cells/l) developed actually in 2.4% of patients treated with abciximab and 0.5% of those treated with tirofiban ($p < 0.001$) in a large series of patients undergoing coronary stenting in the setting of the TARGET study. Here abciximab use was independently associated with the risk of thrombocytopenia

(8). The abciximab (ReoPro) re-administration registry showed that the rate for this complication rises to about 4% after a second exposure to the drug (9). Thrombocytopenia is in some cases accompanied by fever, dyspnea, hypotension, and even frank anaphylaxis, occurring soon after starting the drug (10). Most patients recover uneventfully, but life-threatening bleeding events have been described (11), and several patients have experienced intracranial haemorrhage (12), which is otherwise a much rarer event with this class of drugs. Regardless of the cause, thrombocytopenia was associated with more ischaemic events, bleeds and transfusions in the TARGET trial (8), and was an independent predictor of 30-day mortality in more contemporary data of patients also treated with clopidogrel in the ISAR studies (13). In the large GRACE Registry, collecting data on 52,647 patients with an acute coronary syndrome, patients with specifically defined glycoprotein IIb/IIIa-associated thrombocytopenia were significantly more likely to die in the hospital compared with those without (adjusted odds ratio [OR] 3.45, 95% confidence interval [CI] 2.35 to 5.05), with an adjusted odds ratio numerically higher than that for heparin-induced or other causes of thrombocytopenia (14).

According to the timing related to the administration of the drug, there are two main types of thrombocytopenia associated with the use of abciximab. Most patients develop a fall in platelet count within a few hours of starting therapy with the drug. However, a group of patients, estimated as smaller, has also been described, in whom the drop in platelet count occurred 5–8 days after the drug was administered (15). Paradoxically, plasma of some of such patients induced abciximab-dependent activation of control platelets, leading to aggregate formation. Activating antibodies have since been described also following tirofiban and eptifibatide treatment (12). Thus the immune response to therapy can be responsible for a delayed fall in pla-

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telet count and, on occasion, potential pro-thrombotic consequences, likely amplified by the discontinuation of antithrombotic treatment frequently occurring in the setting of thrombocytopenia.

In addition to these main patterns of abciximab-related thrombocytopenia, a subset of patients are labelled as “thrombocytopenic” despite actually having a circulating platelet count in the normal range. In such cases, low platelet counts obtained with automated counting instruments have been found to be a consequence of the *in vitro* clumping of platelets in blood samples anticoagulated with ethylenediaminetetraacetic acid (EDTA) (16), and such condition is therefore best termed “pseudothrombocytopenia”: diagnosis can here be done repeating a platelet count in blood anticoagulated with citrate.

The development of severe thrombocytopenia in most cases within hours of a patient's first exposure to abciximab is in distinct contrast to most types of drug-induced thrombocytopenia, which occurs in patients who have previously been exposed to the sensitizing drug or have received it for a number of days. Because of such a consideration, non-immune mechanisms (restricting the term to conditions not mediated by an adaptive – mostly antibody-related – immune response) were initially considered as a possible explanation for the acute platelet destruction that is typical of this condition. There have been conflicting arguments in the literature for this (see [12] for a review).

Conversely, direct evidence for the immune destruction of platelets in patients receiving abciximab was provided by studies showing that patients who developed severe thrombocytopenia after a second exposure to the drug all had strong IgG and/or IgM antibodies that reacted with abciximab-coated platelets in a flow cytometric assay (11). Some healthy individuals (even unexposed to the drug) have similar types of antibodies, albeit at a weaker titer (11), but such antibodies (a) usually recognise the papain cleavage site at the C-terminus of the abciximab molecule and can thus be inhibited by Fab fragments, at variance from patients' antibodies (11, 17); (b) usually do not react preferentially – at variance from patients' antibodies – with pla-

telets coated with the intact monoclonal antibody 7E3, from which the specificity-determining sequences incorporated into abciximab were derived (11). The normal occurrence of antibodies reacting with enzymatic cleavage sites in human immunoglobulins has long been appreciated (18), and these are unlikely to cause thrombocytopenia. Conversely, antibodies from patients with abciximab-induced thrombocytopenia recognise either murine sequences incorporated into abciximab (drug-specific antibodies) or conformational changes induced by abciximab on its platelet binding site. This would be the reason why treatment with ligand-mimetic GPIIb/IIIa inhibitors can also lead to acute, severe thrombocytopenia (19, 20), sometimes reported to occur with systemic symptoms such as chills, fever, and hypotension (21). The incidence of drug-induced thrombocytopenia in patients receiving tirofiban or eptifibatid has not been rigorously defined but is probably less than for abciximab. The lack of murine epitopes and the reversible nature of GPIIb/IIIa inhibition, exposing ligand-induced new epitopes on platelets for a shorter time (22), may account for these differences.

In the study on this topic reported in the current issue of *Thrombosis and Haemostasis*, Lajus et al. have investigated a relatively large series (n=18) of patients who became thrombocytopenic after abciximab use out of 639 patients with acute coronary syndromes (estimated incidence 2.8%) (23). The authors have here correlated the evolution of the fall in platelet count (and haemoglobin loss) with the development of abciximab-dependent antibodies. These antibodies were tested with a “classical” monoclonal antibody immobilisation of platelet antigens (MAIPA) technique, detecting human IgG associated with α IIB β 3, and flow cytometry, detecting abciximab-dependent and -independent bound IgG, as well as detecting the expression of surface P-selectin as a marker of platelet activation. In addition, the authors have used a newly developed ELISA, in which wells were pre-coated with α IIB β 3, α IIB β 3 in complex with abciximab, and abciximab alone. Thrombocytopenia was defined as a fall of >50% in platelet count after receiving abciximab; nine patients had a nadir of

<50,000 platelets/ μ l (severe thrombocytopenia). Some of the patients had a classic immediate fall in platelet count; in others, thrombocytopenia also occurred rapidly, but only after abciximab was re-used during rescue therapy. Nine patients (50%) developed a delayed maximum platelet loss, i.e. 5–15 days after receiving abciximab.

One has to appreciate the difficulty in collecting a coherent data set in this relatively rare and inhomogeneous clinical setting. Several considerations can here be made.

First, drug-dependent antibodies were clearly present in most patients with delayed thrombocytopenia, but only in some with clearly defined immediate thrombocytopenia: in the last case, such antibodies mostly occurred after a second abciximab use. This clearly suggests a role for a developing antibody-mediated immune response in delayed thrombocytopenia and in thrombocytopenia occurring upon the re-administration of the drug. How to explain thrombocytopenia occurring early on after drug administration in patients naïve to the drug still remains elusive. Although the authors' results still do not exclude an immune mediation of the fall in platelet count, potential mechanisms still remain very speculative at the moment. Even for late-occurring thrombocytopenia in some patients results in antibody testing were negative: antibody absorption to platelets, the occurrence of non-IgG antibodies (selectively tested here) or non-immune mechanisms may come into play, but it is possible that even delayed thrombocytopenia is not a single pathogenetic entity.

Second, the frequent inability of excess soluble abciximab to block the binding of all drug-dependent antibodies to abciximab- α IIB β 3 complexes suggests that some patients also possessed antibodies able to recognise neo-epitopes formed on α IIB β 3 after its association with the drug (“drug-dependent”, but not “drug-specific”). Since such complexes are also likely occurring with other drugs of the same category, such findings reinforce the hypothesis that only some thrombocytopenias after the use of abciximab are specific for the chimeric nature of this drug, and that conversely, some are related to the target effect of abciximab and similarly acting drugs.

Third, for most thrombocytopenic patients here studied, the presence of drug-dependent antibodies did not lead to long-lasting pathological effects, as the platelet count usually recovered 10–20 days after abciximab infusion. The re-establishment of the platelet count does not go hand-in-hand with a fall in antibody titers, but is likely mostly related to the disappearance of abciximab from the circulation, which may require as long as two weeks or more after the short duration of abciximab treatment (24).

In summary, thrombocytopenia after the use of abciximab is clearly a heterogeneous entity, from the standpoints of clinical presentation, the coexistence or not of platelet activation, and the underlying pathogenesis. One important element for the clinician to bear in mind is that half of the cases here described had a late occurrence, only detectable by monitoring platelet count for at least two weeks after abciximab administration, when in most cases the patient is already discharged from the hospital. It may well be that the previous under-reporting of such a condition was due to not being aware or alerted about such a possibility. As it often happens in medicine, one finds mostly what one looks for.

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