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 αIIbβ3 (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. Abciximab 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 (RGD)-containing cyclic heptapeptide epifibatide (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, epifibatide 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 <100x10^9 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 epifibatide treatment (12). Thus the immune response to therapy can be responsible for a delayed fall in pla-
telet count and, on occasion, potential pro-
thrombotic consequences, likely amplified 
by the discontinuation of antithrombotic 
treatment frequently occurring in the set-
ting of thrombocytopenia.

In addition to these main patterns of ab-
ciximab-related thrombocytopenia, a sub-
set of patients are labelled as “thrombo-
ytopenic” despite actually having a circu-
lating 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 ethylenedia-
minetetraacetic 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 thrombocyt-
ope in most cases within hours of a pa-
tient’s first exposure to abciximab is in di-
tinct contrast to most types of drug-in-
duced 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 me-
diated by an adaptive – mostly antibody-re-
lated – 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 im-
mune destruction of platelets in patients 
receiving abciximab was provided by studies 
showing that patients who devel-
oped severe thrombocytopenia after a sec-
ond exposure to the drug all had strong IgG 
and/or IgM antibodies that reacted with 
abciximab-coated platelets in a flow cyto-
metric 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 recog-
nise the papain cleavage site at the C-ter-
minal of the abciximab molecule and can 
thus be inhibited by Fab fragments, at vari-
ance from patients’ antibodies (11, 17); (b) 
usually do not react preferentially – at vari-
ance 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 
ocurrence of antibodies reacting with 
enzymatic cleavage sites in human immu-
noglobulins has long been appreciated 
(18), and these are unlikely to cause throm-
boctopenia. Conversely, antibodies from 
patients with abciximab-induced throm-
boctopenia recognise either murine se-
quences incorporated into abciximab 
(drug-specific antibodies) or conforma-
tional changes induced by abciximab on its 
platelet binding site. This would be the rea-
son why treatment with ligand-mimetic 
GPIIb/IIIa inhibitors can also lead to acute, 
severe thrombocytopenia (19, 20), some-
times reported to occur with systemic 
symptoms such as chills, fever, and hypo-
tension (21). The incidence of drug-in-
duced thrombocytopenia in patients re-
ceiving tirosiban or epitiabide has not 
been rigorously defined but is probably less 
than for abciximab. The lack of murine epi-
topes 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 Haemosta-
sis*, 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 syn-
dromes (estimated incidence 2.8%) (23). 
The authors have here correlated the evolu-
tion of the fall in platelet count (and hae-
moglobin loss) with the development of 
abciximab-dependent antibodies. These 
antibodies were tested with a “classical” 
monoclonal antibody immobilisation of 
platelet antigens (MAIPA) technique, de-
tecting human IgG associated with αIIbβ3 
and flow cytometry, detecting abciximab-
dependent and -independent bound IgG, 
as well as detecting the expression of sur-
face P-selectin as a marker of platelet acti-
vation. In addition, the authors have used a 
newly developed ELISA, in which wells 
were pre-coated with αIIbβ3, αIIbβ3 in 
complex with abciximab, and abciximab 
alone. Thrombocytopenia was defined as a 
fall of >50% in platelet count after receiv-
ing abciximab; nine patients had a nadir of 
<50,000 platelets/μL (severe thrombocy-
topenia). 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 dur-
ing rescue therapy. Nine patients (50%) de-
veloped 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 de-
layed thrombocytopenia, but only in some 
with clearly defined immediate thrombo-
ytopenia: in the last case, such anti-
bodies mostly occurred after a second ab-
ciximab 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 ex-
plain thrombocytopenia occurring early 
on after drug administration in patients 
naive to the drug still remains elusive. Al-
though the authors’ results still do not ex-
clude 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 pla-
telets, the occurrence of non-IgG anti-
bodies (selectively tested here) or non-im-
mune mechanisms may come into play, but 
it is possible that even delayed thrombocy-
topenia is not a single pathogenetic entity.

Second, the frequent inability of excess 
soluble abciximab to block the binding of 
all drug-dependent antibodies to abcixi-
imb-αIIbβ3 complexes suggests that some 
patients also possessed antibodies able to 
recognise neo-epitopes formed on αIIbβ3 
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 na-
ture 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 titer, 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 underreporting 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.

References