

## Clinical Focus

# Assessment of platelets and the endothelium in patients presenting with acute coronary syndromes – is there a future?

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**A**cute chest pain is one of the most frequent reasons for attending emergency medical care. In the last years, a lot of new markers have been described, measuring various components of the acute atherothrombotic event, and novel biomarkers of endothelial activation, inflammation, coagulation and platelet activation are intensively investigated (1–4).

In acute coronary syndromes (ACS) biomarkers should aid the clinician in different ways: In addition to finding the right diagnosis, biomarkers should discriminate between low and high risk patients, which may need urgent percutaneous coronary intervention (PCI) and more aggressive antiplatelet therapy, and identify non responders to the treatment applied. After the acute event, markers should provide information about the future risk of the patient in order to establish a perfect management following the acute event and an individually “tailored” therapy (e.g. aggressive antiplatelet therapy, tight clinical control, modification of risk factors).

## Endothelial markers in ACS

The endothelium plays a crucial role in the process of atherosclerosis, and improved understanding of the endothelial vascular biology has permitted the development of several markers of normal and activated endothelium. Our article will focus only on the assessment of circulating markers in ACS patients, other techniques, e.g. measuring vasodilation via venous occlusion plethysmography or ultrasound flow-mediated dilatation (FMD), are reviewed elsewhere (5).

### E-selectin, adhesion molecules, thrombomodulin

Endothelial cell activation and endothelial cell-leukocyte interactions are a necessary prerequisite for the initiation of the inflammatory processes that predispose to ACS (1, 6). E-selectin, an endothelial cell-specific protein, intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), which are also expressed by other cell types, promote cell-cell adhesion and arrest (1). Soluble (s) forms of ICAM-1, VCAM-1 and E-selectin have been studied as biomarkers in ACS. Although sICAM-1 levels are elevated within 10 hours after onset of chest pain in ACS and remain elevated for months, studies revealed that sICAM-1 seems to have no immediate use in diagnosis and risk stratification in the acute setting (1). Along this line, studies measuring sE-selectin in ACS patients revealed conflicting results (1, 7). sVCAM-1 is elevated in patients with ACS as compared to stable angina (SA) or healthy controls (1, 7). Furthermore, higher levels of sVCAM-1 on presentation are associated with more in-hospital adverse coronary events and major cardiovascular (CV) events at six months (7–9). However, more prospective studies in an acute clinical setting are needed before sVCAM-1 can be validated as a marker of in-hospital risk.

Thrombomodulin (TM) is another endothelial specific molecule, which can be released from injured endothelial cells. In patients with coronary artery disease (CAD) soluble TM has been associated with the recurrence of CV events (10). However, large, prospective studies are missing.

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### Circulating endothelial cells and endothelial progenitor cells

Measurement of circulating endothelial cells (CECs) and circulating endothelial progenitor cells (EPC) in the peripheral blood is another promising method to assess vascular integrity. While CECs seem to originate from the vessel wall after a pathological insult, EPCs are described to arise from the bone marrow and to be important for repair after vascular damage (11). Two different groups report that high levels of CECs may be a novel predictor of adverse CV events in patients with ACS (12, 13). Recently, a prospective study revealed that CECs levels were significantly higher in patients with ACS as compared with stable CAD and healthy controls and associated with an increased risk of both major adverse cardiac events (MACE) and CV death (14). Although measurement of EPC in stable CAD may have prognostic value, much less is known about EPCs in ACS (15, 16). In summary, these new promising markers are not yet ready for routine clinical use. Major drawbacks are the lack of a uniform methodology, which makes it difficult to interpret different studies,

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complex techniques with the need for specialised personnel and – so far – big prospective studies with ACS patients are missing.

### von Willebrand factor

Among all endothelial markers, von Willebrand factor (VWF) has an exceptional position: it is almost exclusively produced by the endothelium, and thus a marker of endothelial activation or dysfunction. VWF is stored in the Weibel-Palade bodies and is released into the circulation in less than 30 minutes *in vivo* (17). Pathophysiologically, VWF plays a crucial role in platelet adhesion under high shear conditions and supports clotting factor activation by serving as a carrier protein and stabiliser for factor VIII (18). As almost all ACS result from thrombus formation in pre-existing atherosclerotic lesions, VWF may therefore better reflect an acute thrombotic event as compared to other endothelial markers. This is supported by evidence from various trials showing that VWF levels are elevated in ACS as compared to stable CAD patients and healthy controls (18). Patients with myocardial infarction (MI) exhibit higher VWF levels than patients with unstable angina pectoris, and the amount of VWF increase is an independent predictor of short-term adverse clinical outcome in patients with ACS (18–22). Moreover, combined evaluation of VWF and troponin I levels in MI patients provides information about long-term prognosis: high levels are significantly associated with the composite endpoint of death, recurrent angina and revascularisation at the one year follow-up (18, 23). This makes VWF an interesting marker for early identification of high-risk patients after ACS, which may benefit from more aggressive treatment and tight clinical control.

VWF levels show a typical time course during the acute event: In ST-elevation myocardial infarction, levels rise at 24 hours (h), peak at 48–72 h and return to basal values at ~14 days (24). Effective thrombolysis, measured by a patent infarct related artery, blunts the rise in VWF levels (25). The change in VWF levels within 24 h after thrombolytic therapy mirrors successful recanalisation and patients with a patent infarct-related vessel show a significant fall in plasma VWF as compared to those with an occluded vessel (26). Protracted elevation of VWF levels has also been observed in patients requiring rescue PCI after failure of thrombolysis (27).

The ENTIRE-TIMI 23 study showed that in MI patients undergoing fibrinolysis increased VWF elevation at 48–72 h was associated with poor coronary flow at presentation and increased risk of death or MI at 30 days (22). This trial also revealed that those patients apparently benefit from adjunctive treatment with enoxaparin after fibrinolysis as compared to unfractionated heparin (22).

Some MI patients with restored coronary flow after PCI still show abnormal myocardial perfusion, which leads to increased infarct size, reduced left ventricular function and reduced survival (18). VWF release through the PCI procedure itself, especially in multiple coronary stenting, might contribute to this phenomenon (18). Thus assessment of VWF in ACS patients would not only provide information about long- and short-term prognosis of ACS patients but may also serve as a measure for the success of recanalisation therapies and influence the choice of the anticoagulants.

Recently, PRIME, a prospective, population-based study, showed that VWF was associated with ACS, even independent of conventional risk factors (28). On the other hand, other data indicate that plasma VWF levels are at best a weak predictor of CV disease in the general population (17, 29). A more in-depth discussion of these conflicting results is found elsewhere (17, 30).

There is evolving evidence, that VWF is not merely a prognostic marker but rather directly involved in the pathogenesis of ACS, which makes it a possible therapeutic target as well (18). And indeed preclinical testing of experimental VWF antagonists has shown promising results in various models of ischaemia and some of them are now entering early clinical trials (18, 29, 31, 32).

VWF plasma levels are critically dependent on the cleavage enzyme ADAMTS-13. It has been shown that ADAMTS-13 levels are associated with MI risk (33). Unfortunately, all currently available assays are time consuming.

In summary, it seems that VWF, which can be easily measured with commercially available standardised assays, is a promising candidate for routine clinical use in the near future, although still more large prospective studies are needed.

### Platelet function testing in ACS

Recent data provide evidence that not only VWF but also platelet plug formation under high shear rates, as measured by the platelet function analyser (PFA-100®), is predictive of the degree of myocardial necrosis, myocardial blood flow and future events in patients with ACS (29, 34–41). The PFA-100 was originally designed to detect VWF deficiency but is equally sensitive to excess VWF levels (42). It is FDA-approved and determines platelet function under high shear rates. The PFA-100 is a whole blood assay that measures the time for occlusion (closure time, CT) of an aperture in a membrane coated with either collagen and epinephrine (CEPI) or collagen and adenosine diphosphate (ADP; CADP) (42).

However, assessment of platelet function with the PFA-100 can not only aid the clinician to identify high-risk patients but help in monitoring therapy as well. Several studies report a link between CT and occurrence of ischaemic events. Meta-analyses comparing patients who are responsive to aspirin treatment and patients with persistent platelet activity despite aspirin therapy suggest that the latter group faces a significantly (Krasopoulos et al.: nearly 4-fold) greater risk of adverse CV events (35, 43–45). Another meta-analysis, based only on prospective studies, shows that high residual platelet reactivity in aspirin-treated CV patients evaluated by the PFA-100 is associated with recurrent ischaemic events (46). Similarly, a systematic review by Crescente et al. (47) revealed a significant increase in risk of CV events in patients with persistent platelet activity despite aspirin therapy detected by PFA-100.

Therefore it seems that the often criticised “laboratory aspirin resistance” is a clinically important phenomenon, and the PFA-100 device provides an easy to use and rapid test (48). However, standardised cut-off values have to be established and large prospective, randomised clinical trials are needed to determine if PFA-100 testing is useful for making therapeutic decisions, showing that ACS patients with low CEPI-CT and CADP-CT values benefit from more aggressive treatment.

**Table 1: Summary of endothelial and platelet assessment methods discussed in this review.**

Marker	Method of assessment	Technical skills required	Pros/Cons of Method
<b>Endothelial markers</b>			
E-selectin, sICAM-I, sVCAM-I, sTM	ELISA	Specialised lab and personnel	+ : commercially available assays - : not rapid, at the moment not ready for routine clinical use
VWF	ELISA, turbimetric methods	Specialised lab and personnel	+ : commercially available assays - : not rapid enough for ACS diagnosis, at the moment not ready for routine clinical use
CEC	Immunobead method, flow cytometry	Highly specialised lab and personnel	- : diversity of methods, difficult to compare results from different studies; not ready for routine clinical use at the moment
EPC	Cell culture, flow cytometry	Highly specialised lab and personnel	- : like CEC; no good consensus of how to distinguish CEC from EPC
<b>Platelet function</b>			
LTA	Aggregation, optical transmission	Highly specialised lab and personnel	+ : old gold standard, tied to clinical outcomes - : time and extent of processing are inhibitive, lack of standardisation
<b>'Rapid test systems'</b>			
PFA-100	Time for platelet plug formation under high shear conditions	None	+ : easy to use, fast, no sample processing - : depending on VWF, further data for cut-off levels required
VerifyNow P2Y12	Aggregation, optical transmission	None	+ : user-friendly, fast, no sample processing - : unadjustable agonist concentration, further data for cut-off levels required
MEA	Aggregation, impedance	Possibly specialised lab and personnel	+ : physiologic environment, minimal sample manipulation - : less clinical evidence with assay, smaller range for response, further data for cut-off levels required

sICAM-I: soluble intercellular adhesion molecule-I, sVCAM-I: soluble vascular cell adhesion molecule-I, sTM: soluble thrombomodulin, VWF: von Willebrand factor, CEC: circulating endothelial cells, EPC: circulating endothelial progenitor cells, PFA-100: platelet function analyzer-100, LTA: light transmittance aggregometry, MEA: multiple electrode platelet aggregometry ; partly adapted from Oestreich et al (74).

One may criticise that PFA-100 is not platelet-specific but is influenced by VWF (42). On the other hand, this influence might be an advantage of the PFA-100 test in ACS patients: it provides a global assessment of non-vascular primary haemostasis, and this might even better reflect the acute atherothrombotic event than assessment of sole platelet function.

In ACS, a dual antiplatelet therapy with aspirin and clopidogrel has become standard for PCI and stenting (49). However, open questions remain, like what is the optimal loading dose, maintenance dose and duration of therapy for the individual patient; which patient needs even more aggressive antiplatelet therapy with additional substances? Clopidogrel response varies a lot in patients but also in healthy subjects mainly due to genetic differences in metabolising enzymes or drug-drug interactions (50–52). Several studies have shown that the degree of ADP-induced platelet aggregation post-PCI correlates with subsequent ischaemic events and death, and data suggest that relative hyporesponsiveness to clopidogrel increases the risk of stent thrombosis (18, 53–65). Furthermore, several trials revealed that various therapeutic manipulations, including the novel thienopyridine prasugrel, improved antiplatelet response (60). And a recent, prospective small study supports the use of a tailored ap-

proach based on ex-vivo platelet function testing to dose-adjust clopidogrel and reduce ischaemic events following PCI (66).

However, the previous gold standard light transmission aggregometry (LTA), is costly, time consuming and not applicable in an acute clinical setting (60). Moreover, lack of standardised cut-offs makes interpretation of different studies difficult, although a good correlation between several methods and the LTA was shown (60, 67). Two potential candidates to fulfil clinical needs are the Accumetrics VerifyNow Clopidogrel (P2Y<sub>12</sub>) assay (68) and the multiple electrode platelet aggregometry (MEA) (69), which are easy to use and rapid tests appropriate for the acute setting. The MEA implements the principle of impedance aggregometry with no need for blood centrifugation and the ability to assess platelet function in ~ 10 minutes (64). Low response to clopidogrel assessed with MEA was significantly associated with an increased risk of stent thrombosis (64). The Accumetrics VerifyNow is an automated whole blood assay that measures agglutination of fibrinogen-coated beads in response to specific agonists (60, 67). Platelet function measured by VerifyNow is said to identify generalised high platelet reactivity in aspirin-treated patients (70). However, issues regarding the lack of reproducibility of assessment of aspirin responsiveness have been

raised for devices such as the VerifyNow (71). The relationship between results of this point-of-care test and CV events was evaluated in several smaller studies (60, 72). And recently the 3T/2R study revealed that in low-risk patients according to clinical presentation with poor responsiveness to standard oral platelet inhibitors assessed via VerifyNow, intensified platelet inhibition with tirofiban lowers the incidence of MI after elective coronary intervention (73). Additionally, two large trials with this device are currently running, which should provide even more valuable insight into the role of platelet function testing in the setting of PCI (74, 75).

For a summary of endothelial and platelet assessment methods discussed in this review see Table 1. Due to limited space we could only focus on global platelet function assessment in ACS whilst other new, promising platelet activation markers are reviewed elsewhere (2, 6, 76, 77).

## Conclusion

New biomarkers are of clinical value in ACS patients if they are accurate, reproducibly obtained in a standardised fashion and if the procedure is acceptable for the patient and clinicians can easily interpret the results. The ideal biomarkers must have high sensitivity and specificity for the outcome expected, and must also be independent of other risk factors and knowledge of the levels must change patient management (78).

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