Direct oral anticoagulants and antiplatelet agents

Clinical relevance and options for laboratory testing

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Keywords
Oral anticoagulation, platelet aggregation, drug monitoring

Summary
Oral anticoagulants and platelet receptor blockers are widely used in clinical practice with the aim of reducing the risk of thrombotic complications in patients with cardiovascular diseases. Their regular intake and adequate antithrombotic action is vital and this is way numerous assays have been developed for laboratory testing and monitoring of these agents. Available assays can be stratified into pharmacokinetic and pharmacodynamic assays. Such assays are increasingly used in clinical routine and their daily use is triggered by the advent of the novel direct oral anticoagulants (DOACs) as an alternative for vitamin K antagonist (VKA) treatment, which are dabigatran, rivaroxaban and apixaban, and by the advent of prasugrel or ticagrelor as an alternative for clopidogrel with regard to platelet P2Y₁₂ receptor inhibition. In this review the most important and most commonly used laboratory assays are summarized as well as their clinical implications with the focus on DOACs as an alternative for VKAs and the different P2Y₁₂ receptor blockers for antiplatelet treatment.

Oral anticoagulants and platelet receptor blockers are commonly used alone or even in combination for various indications with the sole purpose of reducing the risk of thrombotic complications in patients with cardiovascular diseases. However, for both anticoagulants and antiplatelet drugs the landscape has changed substantially in recent years. Formerly and with regard to anticoagulants, vitamin K antagonists (VKAs) were used in a virtually unrivalled environment. The same situation was true for the use of clopidogrel (commonly administered in combination with aspirin) for P2Y₁₂ receptor directed platelet inhibition.

Now, the situation has changed considerably and is characterized by a number of newly developed and alternative agents for everyday use in the field of anticoagulation and antiplatelet treatment. These agents – commonly termed as direct oral anticoagulants (DOACs) – are dabigatran, rivaroxaban or apixaban for anticoagulation, and prasugrel or ticagrelor for P2Y₁₂ receptor directed antiplatelet treatment.

The availability of these agents is a major medical progress for the treatment of patients with cardiovascular diseases since many limitations of the old agents (e.g. need for INR monitoring with VKAs or low and delayed response with clopidogrel treatment) were overcome by the new drugs. However, physicians are now “spoilt for choice” when choosing between the different agents (1). While certain clinical scenarios demand the use of one over the other agent, in many cases it is nearly impossible to determine the best agent for the individual patient – keeping in mind that

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Pharmacokinetic and pharmacodynamic assessment are key concepts in understanding the behavior of anticoagulants and antiplatelet substances. Pharmacokinetics involves the measurement of drug plasma levels reflecting in-vivo conditions, while pharmacodynamics assesses the ex-vivo measurement of the presumed drug effect.

To interpret the results of laboratory monitoring of antiplatelet and anticoagulant agents, a basic understanding of the different analytical concepts is mandatory:

- **Laboratory testing** of the DOACs is performed by highly specific assays measuring plasma levels of the respective substances.
- Platelet aggregation inhibitors, VKAs or even unfractionated heparin (UFH) are assessed by global testing of platelet aggregation or plasma coagulation.

For the interpretation of laboratory results, different half-life times as well as different dynamics of biological activities of antiplatelet and anticoagulant substances have to be considered. VKAs and antiplatelets exhibit a very long biological activity independent of their pharmacokinetic properties. DOACs and low molecular weight heparins (LMWH) show a comparable half-life time of approximately 12 hours, in which pharmacodynamic and -kinetic coagulation assays parallel each other. UFH and intravenous thrombin inhibitors have to be applied by continuous infusion due to their short half-life times. The important differences of anticoagulants and antiplatelets with regard to PK and PD dynamics are summarized (Table 1).

This review presents the most important and commonly used laboratory methods as well as their clinical implications with a focus on the DOACs as an alternative for VKAs and the P2Y12 receptor blockers for antiplatelet treatment.

### Oral anticoagulants

#### Laboratory testing, drug monitoring

For many years, the terms “oral anticoagulation” and “vitamin K antagonists” have been used synonymously. VKAs exhibit a quite variable and thus unpredictable dose-response relationship related to genetic differences in drug metabolism and to various food and drug interactions. Individual dosing based on laboratory INR testing is mandatory. A strong relationship between the therapeutic quality of treatment (expressed as time in therapeutic range) and outcome has been observed across a large number of studies. However, there remain many reasons for erroneous INR results in clinical routine like pre-analytical conditions, heterogeneity of test reagents and problems with calibration strategies.

DOACs (apixaban, dabigatran, rivaroxaban) are administered on a daily or twice daily standard dosing schedule, with the dosage being mainly determined by indication, age and/or creatinine clearance. There is no routine laboratory testing, although in certain situations (e.g. emergency, perioperative, compliance testing) pharmacokinetic and/or -dynamic measurements need to be available.

For UFH, VKAs and intravenous direct thrombin inhibitors anticoagulant monitoring in clinical routine is performed by global coagulation assays giving the sum of the patient’s intrinsic haemostatic potential together with the functional impact of anticoagulants present in plasma. Intravenous agents like UFH or thrombin inhibitors are commonly applied in emergency situations and need an individual monitoring based on aPTT clotting assays, which also reflect the patient’s haemostatic potentials together with the plasma levels of the respective compound. Therapeutic ranges of aPTT and activated clotting time (ACT) have been established for UFH and intravenous thrombin inhibitors.

The laboratory monitoring of vitamin K antagonists is independent of the plasma levels of the drug. The effect of the medication on the synthesis of partly defect coagulation factors is measured by the prothrombin time (PT) and reported in international normalized ratio (INR).

### Tab. 1 Application, dynamics and pharmacological monitoring of antiaggregants and anticoagulants

<table>
<thead>
<tr>
<th>monitoring, pharmacological properties</th>
<th>anticoagulant or antiaggregant</th>
</tr>
</thead>
<tbody>
<tr>
<td>pharmacokinetic (PK)</td>
<td>ASS</td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>pharmacodynamic (PD)*</td>
<td>aggregation in liquid phase or on surfaces</td>
</tr>
<tr>
<td>rapid change in PK/PD</td>
<td>---</td>
</tr>
<tr>
<td>application</td>
<td>p.o. or i.v.</td>
</tr>
</tbody>
</table>

PT: prothrombin time; INR: international normalized ratio; aPTT: activated partial thromboplastin time; ACT: activated clotting time; TT: thrombin time; ECT: ecarin clotting time; * see Table 2 for details with regard to DOACs and global coagulation testing.
It is impossible and even misleading to transfer the traditional anticoagulant monitoring using pharmacodynamic models like INR to DOACs.

Therefore, measuring plasma levels of DOACs has been introduced in clinical routine using highly specific clotting or chromogenic assays (8–10). The rapid change of peak and plasma concentrations within the 12 or 24 hours dosing regimen has to be taken into account. Global and specific coagulation assays can be discriminated with respect to the possible influence of matrix components of the blood sample. Sample dilution, adding cofactors or target enzymes to the reagent mixture as well as using synthetic substrates attenuates the influence of coagulation factors, interfering drugs and other matrix compounds, which on the other hand impact on global coagulation assays. The differences between pharmacodynamic and -kinetic testing of anticoagulants is shown (Fig. 1).

**Clinical relevance of laboratory testing**

The concept of the therapeutic range in VKA administration has been developed and validated for years, finally leading to the global acceptance of the INR concept and related therapeutic windows (11). In recent years, the assessment of the quality of VKA administration has been discussed intensively. A strong association between the time in therapeutic range (TTR) and bleeding or thromboembolic complication has been observed across a large number of studies (4). The time in therapeutic range is highly dependent on the time period since the start of treatment, as could be demonstrated in a recent systematic review and meta-analysis regarding VTE treatment (12).

Surprisingly, all DOAC studies in atrial fibrillation patients showed relatively low numbers of TTR in the VKA groups (13). Concerning DOACs, missing sensitivity, linearity and inconsistent clinical interpretation of results of global coagulation assays complicate the situation although for some PT and aPTT reagents DOAC-plasma level dependent prolongation of the respective clotting times has been shown. As a first solution PK assessment has been introduced using diluted thrombin time, anti-factor IIa and anti-factor Xa testing (Tab. 2). Recent data indicate an association of trough levels of dabigatran with efficacy and safety outcome (14). Thus, therapeutic drug monitoring seems to be a feasible concept for laboratory assessment of the DOACs in emergency situations. However, exact knowledge of the time point of drug intake and blood withdrawal is a basic prerequisite for interpretation of results. Moreover, manufacturers for each compound have to provide specific peak and trough level data as measured in the large clinical trials. More controlled and prospective studies are urgently needed here to link peak and/or trough levels ob-

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**Fig. 1** Pharmacodynamic vs. pharmacokinetic testing for anticoagulant: influence of blood sample selection and preparation (whole blood, plasma, diluted plasma) and analytical concept (clotting, synthetic substrate assay) on sensitivity and specificity of coagulation tests for anticoagulant monitoring (ACT: activated clotting time; PT: prothrombin time; aPTT: activated partial thromboplastin time; ECT: ecarin clotting time; dil. TT: diluted thrombin time)

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**Tab. 2**

Laboratory assays for monitoring of the direct oral anticoagulants

<table>
<thead>
<tr>
<th>test</th>
<th>dabigatran</th>
<th>rivaroxaban</th>
<th>apixaban</th>
</tr>
</thead>
<tbody>
<tr>
<td>global coagulation assay</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>prothrombin time / INR</td>
<td>insensitive</td>
<td>dose dependent increase, variable sensitivity</td>
<td>dose dependent increase, low sensitivity</td>
</tr>
<tr>
<td>activated partial thromboplastin time</td>
<td>variable, non-linear sensitivity</td>
<td>dose dependent increase (low sensitivity)</td>
<td>dose dependent increase (weak sensitivity)</td>
</tr>
<tr>
<td>activated clotting time</td>
<td>variable sensitivity</td>
<td>weak sensitivity</td>
<td></td>
</tr>
<tr>
<td>drug monitoring</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>anti-Xa assay (synthetic substrate)</td>
<td>NA</td>
<td>sensitive quantification of plasma level</td>
<td></td>
</tr>
<tr>
<td>diluted TT</td>
<td>sensitive quantification of plasma level</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>ecarin clotting time</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

INR: international normalized ratio
Laboratory-based methods like light transmission aggregometry (LTA) and the vasodilator-stimulated phosphoprotein (VASP) assay lack standardization (esp. for LTA), require skilled personnel, are time-consuming and do not allow for a near-patient testing. Of note, for the VASP assay a new ELISA-based method allowing for easier and rapid testing has been developed just recently (17). On the other hand, near-patient or point-of-care tests like the VerifyNow assay or the Multiplate analyzer are highly standardized, rapid and less laborious with regard to test performance and in consequence, they can be more easily incorporated into routine clinical practice. Concerning standardization of assays, it is also important to realize that only for standardized assays cut-off values for both thromboembolic and bleeding risk can be defined, that can be used all over the place with the aim of an individual risk stratification for both clinical entities. The characteristics of the most commonly used assays are summarized (Tab. 3). A detailed explanation of available tests and how they are performed is beyond the scope of this manuscript and described elsewhere (18, 19).

### Clinical relevance of laboratory testing

The relevance and importance of platelet function testing needs to be considered in view of the perspectives and scenarios in which testing can be used in clinical practice. Three completely different perspectives are important here.

<table>
<thead>
<tr>
<th>Method</th>
<th>Test</th>
<th>Medium</th>
<th>Detection</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory based</td>
<td>light transmission aggregometry (LTA)</td>
<td>platelet-rich plasma (PRP)</td>
<td>reduction of optical density after stimulation in PRP</td>
<td>instrument adjustment possible, good predictivity, long experience</td>
<td>time consuming, complex sample preparation, no standardization</td>
</tr>
<tr>
<td></td>
<td>vasodilator-stimulated phosphoprotein (VASP) assay</td>
<td>whole blood</td>
<td>flowcytometric detection of VASP phosphorylation</td>
<td>whole-blood assay, longer sample storage, P2Y12 receptor specific</td>
<td>very time consuming, complex sample preparation, need of a flowcytometer</td>
</tr>
<tr>
<td></td>
<td>thrombelastography (TEG) platelet mapping assay</td>
<td>clot formation</td>
<td>whole-blood assay</td>
<td>complex and time consuming procedure, requires pipetting, limited experience and study results with P2Y12 inhibitors</td>
<td></td>
</tr>
<tr>
<td>Near patient</td>
<td>multiplate analyzer (MEA)</td>
<td>whole blood</td>
<td>coating of 2 electrode pairs by platelets</td>
<td>whole-blood assay, simple and rapid standardized procedures</td>
<td>semi-automated (requires pipetting), rapid processing of samples necessary</td>
</tr>
<tr>
<td></td>
<td>VerifyNow</td>
<td>platelet-mediated aggregation of fibrinogen-coated polystyrene beads</td>
<td></td>
<td>no assay adjustment possible, expensive cardridges</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PFA 100</td>
<td>closure of an aperture of a collagen-coated membrane</td>
<td></td>
<td>no assay adjustment possible, dependent on haematocrit and VWF, limited experience and study results with P2Y12 inhibitors</td>
<td></td>
</tr>
</tbody>
</table>
Fig. 2  Studies and evidence for the existence of “therapeutic window concept” of P2Y12 receptor inhibition according to Tanty et al. (18), based on data obtained by a) VerifyNow P2Y12 assay; b) VASP assay; c) Multiplate analyzer; d) Thrombelastography Platelet Mapping Assay

Tab. 4  Studies investigating the therapeutic window concept of P2Y12 receptor inhibition (22–27)

<table>
<thead>
<tr>
<th>first author</th>
<th>patients (n)</th>
<th>assay</th>
<th>patients’ condition</th>
<th>drug</th>
<th>therapeutic window (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bonello</td>
<td>301</td>
<td>VASP</td>
<td>acute coronary syndrome</td>
<td>prasugrel</td>
<td>PRI VASP: 16–53.5%</td>
</tr>
<tr>
<td>Campo</td>
<td>300</td>
<td>VerifyNow P2Y12</td>
<td>all comers (except STEMI)</td>
<td>clopidogrel</td>
<td>PRU: 86–238</td>
</tr>
<tr>
<td>Cuisset</td>
<td>1542</td>
<td>VASP</td>
<td>acute coronary syndrome</td>
<td>clopidogrel &amp; prasugrel</td>
<td>PRI VASP: 10–50%</td>
</tr>
<tr>
<td>Gurbel</td>
<td>225</td>
<td>TEG platelet mapping assay</td>
<td>acute coronary syndrome</td>
<td>clopidogrel</td>
<td>MA ADP: 31–47 mm</td>
</tr>
<tr>
<td>Mangiacapra</td>
<td>732</td>
<td>VerifyNow P2Y12</td>
<td>stable CAD</td>
<td></td>
<td>PRU: 179–238</td>
</tr>
<tr>
<td>Sibbing</td>
<td>2533</td>
<td>Multiplate analyzer</td>
<td>all comers</td>
<td></td>
<td>AU x min: 189–467</td>
</tr>
</tbody>
</table>

VASP: vasodilator-stimulated phosphoprotein; PRI VASP: platelet reactivity index VASP; ADP: adenosine diphosphate; CAD: coronary artery disease; PRU: P2Y12 reactivity units

Diagnostic level

Platelet function testing can be used as a diagnostic marker to determine the level of on-treatment platelet reactivity at a specific time point for the individual patient (diagnostic level of testing). This can be useful in a peri-operative setting when the agent is paused and the offset of the antiplatelet action needs to be confirmed before an operation is started. In addition, diagnostic testing can also be of use after a clinical event (e.g. stent thrombosis) occurred and a work-up in search for possible reasons is mandatory.

Prognostic level

Testing results can be used as a biomarker to define the prognosis of the individual patient after a certain procedure (e.g. coronary stenting) has been performed (prognostic level of testing). Accumulating data from a number of large studies in >20000 patients emphasize the importance of high on-treatment platelet reactivity (HPR) especially with regard to P2Y12 receptor stimulation as a prognostic risk factor (18, 19).

Just recently, the ADAPT-DES (Assessment of Dual AntiPlatelet Therapy with Drug-Eluting Stents) trial involving 8575 patients reinforced the independent association between HPR and the risk for stent thrombosis (20). Observational data on the prognostic value of testing for predicting ischaemic events can be found elsewhere in detail (18, 19).

Beyond the risk prediction for ischaemic event, recent observational studies have also highlighted the association of an enhanced drug response (= low on-treatment platelet reactivity) with the risk for bleeding events (18, 19). The importance of bleeding risk is emphasized by the circumstance that similar as for ischaemic events bleeding complications are associated with overall mortality and especially the newer antiplatelet agents prasugrel and ticagrelor are both associated with a higher risk for bleeding. Using the combined information that platelet function testing can deliver for both ischaemic and bleeding risk prediction, the ultimate aim must be to define a therapeutic window of P2Y12 receptor di-
rected platelet inhibition (21), similar as it is widely established with the INR value that clearly defines the borders of desired anticoagulation delivered by VKAs.

Evidence is accumulating that a therapeutic window (Fig. 2) or “sweet spot of platelet inhibition” also exists for P2Y12 receptor inhibition. Data from studies that focus on the therapeutic window concept (22–27) are shown (Tab. 4). Further studies are needed to support this concept and transfer it into routine clinical practice.

Guidance level

While the value of testing on a diagnostic and prognostic level is widely accepted, the value of testing for guidance of a tailored antiplatelet treatment (guidance level of testing) is a topic of ongoing debates. Previous randomized clinical trials (GRAVITAS, TRIGGER-PCI and ARC-TIC) using the VerifyNow assay were not supportive for an individualized treatment approach of P2Y12 receptor directed platelet inhibition (28–30). However, these studies had little utilization of potent antiplatelet agents and included mainly stable patients undergoing PCI, a cohort of patients where routine PF testing with adaptation of treatment may be of limited value as compared to high-risk patients undergoing coronary stenting (20). Of note, smaller studies using different devices for PF testing such as the VASP assay or the Multiplate analyzer provided promising results (31, 32). Studies investigating the value of PF testing for guidance of treatment in ACS patients and with a focus on both ischaemic and bleeding events are still lacking. Moreover, the value of testing for guidance of treatment in the sub-acute and chronic phase of ACS patients undergoing PCI remains to be explored.

The TROPICAL-ACS (Testing Responsiveness to Platelet Inhibition on Chronic Antiplatelet Treatment For Acute Coronary Syndromes) trial (ClinicalTrials.gov Identifier: NCT01959451) aims at closing these gaps of knowledge. The primary objective of the trial will be to investigate whether a platelet function testing guided approach with a short term (1 week) prasugrel treatment and a switch-over to clopidogrel treatment in adequate responders to clopidogrel is non-inferior in terms of thrombotic and bleeding risk to a 12 month standard treatment with prasugrel in ACS patients treated with PCI. Enrollment into this phase IV, prospective, randomized, parallel-group, open-label, non-inferiority, multi-center trial is planned to start at the end of 2013 with a planned enrollment of 2600 patients in about 20 investigational centres in Europe.

Actually, the concept of tailored antiplatelet treatment and its possible benefits remains unproven based on the results of the large-scale clinical trials (28–30). However, it cannot be deemed as disproven due to the obvious limitations of these trials (27–29).

Conclusions

Treatment with anticoagulants and anti-aggregatory agents as well as their laboratory monitoring share common features. Understanding the pharmacokinetic and pharmacodynamic concepts in coagulation and platelet function testing is of utmost importance for a correct interpretation of testing results.

Correct interpretation of results and the clinical implications that come along with them have to be established in close communication between laboratory doctors and clinicians. The following aspects must be taken into account when dealing with the different agents and their laboratory drug monitoring:

- Platelet receptor blockers as well as VKAs are monitored by pharmacodynamic assays (platelet function testing, prothrombin time or INR).
- A therapeutic window of coagulation treatment is well established for VKAs, while evidence is accumulating that such a “sweet spot” also exists for P2Y12 receptor directed platelet inhibition.
- The novel oral anticoagulants cannot be precisely monitored by conventional pharmacodynamics assays and must be evaluated by specific pharmacokinetic monitoring making the time of drug intake and time of blood drawing an inevitable information for interpretation of test results.
- Pharmacokinetic assays are characterized by a high sensitivity and specificity for monitoring the respective agents.
- Pharmacodynamics assays are influenced by intrinsic plus extrinsic factors and the direct action of the drug.

Conflict of interest

The authors declare that they have no conflict of interest.

References


