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ZIEKENHUIS ST ANTONIUS

Anti-platelet therapy in (cardio)vascular disease

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Recent years have witnessed significant advances in the treatment of patients with atherosclerotic (cardio) vascular disease and dual anti-platelet therapy with aspirin and clopidogrel (an ADP P2Y12 receptor antagonist) has become the cornerstone in the acute and long-term management of patients with coronary, cerebral and peripheral artery disease. Aspirin and clopidogrel interfere with platelet activation in complementary, but separate pathways. The result is an even stronger anti-platelet effect translating into superior antithrombotic protection without an increase in bleeding complications.

A number of clinical trials have demonstrated the incremental benefit and efficacy of the combination of clopidogrel and aspirin therapy above and beyond that of aspirin alone. However, it has been demonstrated that the pharmacological response to clopidogrel is not uniform in all individuals and that low-response and/or non-response is associated with an increased risk of adverse outcomes, of which stent thrombosis is the most feared. Consequently, evaluation of the pharmacological response to antiplatelet therapy by monitoring platelet function inhibition has become an new field of interest and probably improves patients outcome. However, the road to platelet inhibition by clopidogrel is bumpy and the determination of the best measurement of clopidogrel response is a challenge.

Clopidogrel is absorbed as a prodrug in the intestine by P-glycoprotein, after which 85 % is inactivated and only 15 % is metabolized through the intermediate 2-oxo-clopidogrel to it's active metabolite. This two-step metabolization process has been proposed to be mediated by CYP's 2C19, 2C9, 3A4, 3A5. The active metabolite irreversibly binds to the P2Y12 receptor, in which several Single Nucleotide Polymorphisms (SNP's) that possibly contribute to a decreased affinity for clopidogrel, are known. Finally, this blockade of the P2Y12 receptor leads to decreased platelet response to ADP. As the response of normal subjects to ADP has a wide variety, one might expect a large degree of variation in on-clopidogrel platelet function as well. We hypothesized that the only way to take all these variables into account is the endpoint of the mentioned process, platelet function measurement. To link this marker for clopidogrel response to adverse clinical outcome we designed the POPular study.

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POPular study
In the POPular study 1068 consecutive patients scheduled for elective Percutaneous Coronary Intervention (PCI) with stent placement were included (1). After adequate clopidogrel treatment blood was drawn after which eight different platelet function tests were performed. After the procedure, patient follow-up was performed after 3 and 12 months and the predefined primary endpoint (death, stent thrombosis, myocardial infarction and CVA), as well as the primary safety endpoint (bleeding) was scored. By ROC curve analysis we defined the optimal cut-off for each test splitting the population in patients with 'high on treatment platelet reactivity' (HPR) and 'normal on-treatment platelet reactivity' (NPR). With this design we proved able to use platelet function as a predictor for clinical outcome. Light transmittance aggregometry (LTA), the VerifyNow POC whole blood test, the PlateletWorks single platelet disappearance assay and the novel PFA100 P2Y* were independent predictors for the occurrence of events. The Odds Ratio for suffering an event on HPR was similar as clinical risk factors like low cardiac output (LVEF<45%), renal failure, prior CABG and age. Although the results seemed promising, our initial goal to predict the future occurrence of events was not achieved with PPV's of 11-14%, leaving the results especially promising as an excluder of events (NPV's 90-95 %)**.** This was accounted for by the relatively low event rate of this stable patient selection.

MAPCAT study
Initiated from our group, an evaluation of the occurrence for stent thrombosis in five Dutch PCI centers was performed. This cohort was approached to perform a reverse analysis compared to the POPular study, called the MAPCAT study (2). Patients with a history of stent thrombosis (ST) were subjected to four platelet function tests (LTA, VerifyNow, PFA P2Y* and VASP-phosphorylation), after which they were loaded with clopidogrel and subsequently the tests were repeated. Also, at eight time points between 0-8 hours blood was sampled for measurement of clopidogrel metabolites. The cohort consisted of patients with early ST (< 30 days after PCI, n=41) and late ST (> 30 days after PCI, n=43). A cohort of 103 patients who underwent PCI without ST served as a control group. As expected, the platelet reactivity before loading with clopidogrel was not significantly different between groups, and the major part of patients were above the cut-off for HPR. Strikingly, after loading with clopidogrel the control group as well as the late ST group showed a good response to clopidogrel, with most patients exhibiting an on-treatment NPR. The group of early ST showed a significantly higher on-treatment platelet reactivity. Using the POPular defined cutoffs, we observed HPR in 25 % of the control group, 63 % in the early ST group and 41 % in the late ST group. This is in line with the hypothesis that poor response to clopidogrel manifests especially in the first month after the procedure.

Pharmacogenetics
In a subanalysis of the TRITON-TIMI38 cohort a major role was postulated for CYP2C19 in the metabolism of clopidogrel, as carriers of the loss-of-function allele CYP2C19*2 had a worse clinical outcome than non-carriers (3). In the POPular cohort we showed that besides the worse clinical outcome CYP2C19*2 was indeed associated with a higher on-treatment platelet reactivity. A less pronounced but also significant association was observed for CYP2C9*3 (4). As a proof-of-principle, both loss-of-function alleles proved to be associated with ST in the MAPCAT cohort (5).
In a close collaboration with the University of Cologne, paraoxonase-1 (PON-1) was identified as a major determinant of clopidogrel metabolism. PON-1 expressed in microsomes was not able to convert clopidogrel to it's intermediate metabolite, but showed a high activity in the conversion of the intermediate to the active metabolite. This activity was significantly lower in the PON-1 R192Q genotype. PON-1 activity correlated well with the active metabolite measured in the MAPCAT cohort. Significantly higher odds ratio's for suffering an cardiovascular event were found in patients with the QQ genotype compared to the RR genotype (6).

Co-medication
As CYP2C19 has a major role in metabolization of medication, the simultaneous prescription of specific drugs might reduce the availability of active components. Indeed, the FDA prohibited the co-prescription of the proton pump inhibitor (PPI) omeprazole with clopidogrel, because this dramatically lowered the concentration of active metabolite and platelet inhibition. From our MAPCAT cohort it became clear that esomeprazole had a smilar effect on the availability of active metabolite and the degree of platelet inhibition, whereas pantoprazole did not interfere with clopidogrel's conversion process (7). Also the use of calcium channel blockers was shown to interfere.

P2Y12 receptor polymorfisms
The P2Y12 receptor is known for several SNP's that might affect the affinity of clopidogrel. In collaboration with the ErasmusMC we determined whether different combinations of SNP's defined in six haplotypes were associated with different platelet reactivity profiles. Using the POPular cohort, we observed a difference up to 8 % platelet reactivity between different haplotypes (8). However, an association of the same haplotypes with the primary endpoint as defined in the POPular study was not found (9).

Current research focus
Although the elective PCI's seem to have a rather low event rate in our center, the identification of patients at risk still is a major win for patient care. Momentarily we are developing a risk score including platelet function, pharmacogenetics and clinical risk factors. Patients exhibiting a high risk will be put on the more stringent P2Y12 receptor antagonist prasugrel. In parallel we focus on patients with acute myocardial infarction, as the advantage of adequate antiplatelet therapy in this category has shown to be even more crucial.

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Rijnstate

Organisatie van het fertiliteitslaboratorium: bekende techniek samenhangend met herkenbare menselijke vragen

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In de fertiliteitskliniek met een laboratorium dat ivf-behandeling, gameet en/of embryodonatie ondersteunt komen uiteenlopende facetten met betrekking tot de hulp van patiënten en cliënten met kinderwens bijeen: laboratoriumtechnische zaken, inrichting, organisatie, procesbeschrijving en risicomanagement, behandelingskwesties en menselijke, psychologisch-ethische vraagstukken. Verantwoorde zorgverlening vergt aandacht voor elk van deze aspecten en oog voor hun onderlinge samenhang. De voortplantingsgeneeskunde is een multidisciplinaire activiteit bij uitstek, waar met kennis en in goede samenspraak een voor alle betrokkenen bevredigend resultaat wordt nagestreefd. Van het fertiliteitslaboratorium mag worden verwacht dat op elk niveau wordt meegedacht en dat de processen zijn ingericht zodanig dat optimaal veilige en verantwoorde dienstverlening wordt verleend.

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Het fertiliteitslaboratorium is in de wereld van de klinische laboratoria een bijzonder geval. Waar het merendeel van het werk in het klinische laboratorium erop gericht is onderzoeksresultaten te produceren, levert het fertiliteitslaboratorium behalve dat ook een 'product' bedoeld voor behandeling: gameten en - als een ivf-laboratorium deel uitmaakt van het fertiliteitslaboratorium - ook embryo's. Het fertiliteitslaboratorium is in dit opzicht te vergelijken met het transfusielaboratorium, dat ook producten voor behandeling uitgeeft.

De uitgifte van biologisch materiaal bedoeld voor behandeling vraagt, vergeleken met het laboratoriumwerk waarbij metingen worden gedaan, om extra organisatie en voorzorgen. Want met gameten wordt nageslacht 'geproduceerd'. Via het toegepaste materiaal kunnen mensen ziekte of schade oplopen. In de eerste plaats geldt dat voor de behandelde personen, de vrouwen (en indirect ook de partners die bij hun wel en wee, en de nakomelingen betrokken zijn). Maar niet minder betreft dat het beoogde nageslacht. Men name wat betreft het nageslacht kunnen eenmaal gemaakte fouten onomkeerbare gevolgen hebben, wat de risico's des te