Antiplatelet effects of clopidogrel and aspirin after interventional patent foramen ovale/atrium septum defect closure

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Abstract
The optimal antiplatelet therapy after patent foramen ovale (PFO)/atrium septum defect (ASD) closure is a matter of discussion. It is challenging as inter-individual responses to antiplatelet medication vary significantly and common complications are bleeding and ischemic events. In this study, we aimed to analyze the incidence of high on-treatment platelet reactivity (HTPR) to antiplatelet medication in patients undergoing PFO/ASD closure as well as clinical complications and thrombus formation on the occluder during six-month follow-up. This hypothesis generating pilot study was observed, which included 140 patients undergoing PFO/ASD closure. The primary endpoint was pharmacodynamic response to antiplatelet medication. A composite of death, myocardial infarction, bleeding, stroke and thrombus formation on the occluder during six-month follow-up was the secondary endpoint. HTPR to clopidogrel was analyzed using the vasodilator-stimulated protein phosphorylation (VASP), HTPR to aspirin by light-transmission aggregometry (LTA). In 71% of patients HTPR to clopidogrel was detected, HTPR to aspirin in only 4%. We observed 12 complications, 9 bleeding events (including 3 major bleeding events) and 3 transient ischemic attacks. No stroke and no thrombus formation on the occluder occurred. The primary endpoint was not associated with the secondary endpoint. The incidence of HTPR to clopidogrel in PFO/ASD closure patients is very high. Despite this high incidence, no stroke or thrombus formation on the occluder occurred at all. This leads to the hypothesis, that the benefit of additional clopidogrel medication is questionable and has to be investigated in large-scale clinical trials.

Introduction
Interventional closure of symptomatic atrial septal defect (ASD) and patent foramen ovale (PFO) has become a standard procedure with excellent technical results. However, the optimal antiplatelet therapy for patients undergoing PFO/ASD closure is controversial since common peri-interventional complications are not only ischemic events and thrombus formation on the closing device but also bleeding. At the moment, different regiments of aspirin (acetylsalicylic acid, ASA) and clopidogrel therapy are applied though there is no decisive data and therefore no definite recommendation. This problem is underlined by the variable dosage and duration strategies of antiplatelet medication within the recent randomized trials (Closure I, RESPECT, PC-Trial) [1–3]. Furthermore, the inter-individual response to aspirin and clopidogrel is highly variable. Impaired pharmacodynamic response to antiplatelet medication is called high on-treatment platelet reactivity (HTPR) [4]. Increased incidence of mortality and stent thrombosis has been described in patients with HTPR undergoing percutaneous coronary intervention (PCI) [5]. HTPR to clopidogrel is likewise associated with a higher risk of stent thrombosis and myocardial infarction after PCI. Accordingly, bleeding complications seem to be more frequent in patients with clopidogrel low on-treatment platelet reactivity (LTPR) [6].

In the present study, we investigated (i) pharmacodynamic response to antiplatelet medication after interventional PFO/ASD closure, (ii) clinical complications and thrombus formation on the occluder during six month follow-up as well as (iii) an association between the incidence of overall clinical complications and antiplatelet effects.

Methods
Patients
Data of 140 patients with interventional PFO/ASD closure between the years 2009 and 2013 at the University Hospital Düsseldorf in Germany have been analyzed. One hundred eight patients underwent PFO closure and 32 ASD closure.

Study design
We performed an observational, monocentric investigation in a real world cohort. The primary endpoint was pharmacodynamic response to antiplatelet medication. The secondary endpoint was the incidence of clinical complications and thrombus formation on the closing device during hospital stay and six months follow-up. The study was approved by the ethics committee of the Heinrich-Heine University Düsseldorf.

Interventional PFO/ASD closure
Closure of PFO and ASD was performed according to the instructions for use. Occluders were provided by St. Jude Medical.

Keywords
ASD, aspirin, clopidogrel, high on-treatment platelet reactivity, PFO

History
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(Helsingborg, Sweden) (19%). Vascular access was achieved by a 9-French delivery system that was extracted manually. No vascular closing device was applied.

**Peri-interventional antiplatelet/anticoagulation regimen**

Patients were administered a loading dose of 600 mg clopidogrel and 500 mg aspirin prior to interventional PFO/ASD closure independent of existing antiplatelet medication. During the intervention, unfractionated heparin (60 I.U./kg) was used to control anticoagulation and keep the activated clotting time between 250 and 300 s. In patients without permanent oral anticoagulation, only thrombosis prophylaxis with unfractioned-, respectively low-molecular weight-heparins was performed starting the day after intervention. Nineteen patients were on oral anticoagulation because of atrial fibrillation or previous pulmonary embolism. Oral anticoagulation was stopped five days before the intervention and discontinued at least until two days after the closure. Unfractionated or low-molecular weight heparin was used for bridge-to-intervention with a 1.5-2 fold increased partial thromboplastin time (PTT) for unfractioned heparin. Low-molecular weight heparin was administered weight adjusted. Heparin administration was started again four hours after withdrawal of the vascular access system. In case of PTT above or below target area, repetitive heparin dose adjustments and PTT measurements every five hours were conducted until the PTT reached the target area. During platelet function analyses, no oral anticoagulants had been administered.

**Postinterventional antiplatelet/anticoagulation regimen**

Beginning with the day of the procedure a daily dose of 75 mg clopidogrel and 100 mg aspirin was administered as dual antiplatelet therapy (DAPT) in 118 patients (84.3%). Fifteen of the 19 patients on oral anticoagulation received additional DAPT (10.7%), four received only additional clopidogrel (2.9%). Application of clopidogrel alone was performed in 3 patients (2.1%).

**Primary endpoint (platelet function analyzes)**

After the PFO/ASD intervention pharmacodynamic response to antiplatelet medication was tested in all 140 patients during hospitalization. Blood withdrawal was conducted using a 21 G needle, three hours after intake of morning medication and two days after administration of aspirin and clopidogrel loading dose. Blood resting was performed for 15 minutes. Clopidogrel antiplatelet effects were analyzed using the vasodilator-stimulated protein phosphorylation (VASP) assay. The VASP test is the most specific way to determine clopidogrel HTPR/LTPR as it inhibits the ADP dependent P2Y12 receptor and therefore specifies the measurement of P2Y12 blockage [7]. Platelet reactivity index (PRI) > 50 was defined as HTPR, while PRI < 16 was designated as LTPR. Aspirin antiplatelet effects were determined by light-transmission aggregometry (LTA) using arachidonic acid (AA) to evaluate platelet reactivity. AA was applied as it allows most definite investigation of inhibited platelet function due to aspirin [9]. Aspirin HTPR was defined as aggregation > 20%.

**Secondary endpoint (adverse events)**

The secondary endpoint was a composite of death, myocardial infarction, major/minor stroke, transient ischemic attack (TIA), thrombus on occluder, life threatening bleeding, major/ minor bleeding and major/minor vascular complication. The bleeding and ischemic complications were specified following the definitions of the updated valve academic research consortium (VARC-2) [10]. Three and six months after intervention follow-up visits were performed. Transesophageal echocardiography (TEE) was conducted during the first follow-up visit and transthoracic echocardiography during the second follow-up visit to evaluate occluder position and possible thrombus formation on the occluder.

**Statistical analyses**

GraphPad Prism©- (LaJolla, CA, USA) and the IBM SPSS©- Software (New York, NY, USA) were used for statistical analyses. Type I error below 0.05 was considered significant. Data are mean ± SD. Gaussian distribution was tested using q–q plots and histograms. No outliers were removed. For comparison of continuous variables the t-test was applied, whereas binary variables were investigated with the chi-squared test. Calculation of the odds ratio was performed according to Altman.

**Results**

**Patient characteristics**

At the date of intervention the patients were 52 ±14 years old. 63 of the 140 patients were of male gender (45%). There were 106 PFO closures and 34 ASD closures. Indication for PFO closure was always secondary preventive (96.2% after TIA/stroke; 3.8% after diving accidents). Intervention in ASD patients was proceeded secondary prophylactic (17.6% after TIA/stroke, 5.9% after diving accidents) or for hemodynamic reasons (76.5%). The occluder was implanted successfully in all 140 patients (100%). 15 patients (11%) additionally suffered from coronary artery disease, 10 (7%) had known atrial fibrillation prior to the intervention. Clinical characteristics are presented in Table I.

**Primary endpoint**

We evaluated platelet reactivity in 140 patients undergoing PFO/ ASD closure. VASP assay measuring clopidogrel antiplatelet effects showed a broad variance with a mean PRI of 66.5 ± 26. (Figure 1) 99 patients (70.7%) displayed clopidogrel HTPR (PRI > 50) while seven (5%) had clopidogrel LTPR (PRI < 16). Mean
aggregation levels for aspirin measured by LTA were 10.2 ± 12%. In five patients (3.8%) aspirin HTPR (aggregation > 20%) was detected (Table II). HTPR to clopidogrel was more frequent in male patients (51 vs. 12 patients; p = 0.02). Besides that, no association between clinical characteristics and incidence of HTPR existed (Supplemental 1).

Secondary endpoint

A composite endpoint of death, myocardial infarction, ischemic/ thrombotic events, and major/ minor stroke, transient ischemic attack (TIA), thrombus on occluder, life-threatening bleeding, major/ minor bleeding and major/ minor vascular complication was observed in twelve patients. No death, stroke, periprocedural myocardial infarction or vascular complication were detected. Also, no thrombus formation on the occluder was found. Nine of the twelve secondary endpoints occurred during hospitalization and three were observed during six-month follow up. The majority of complications were bleeding events (9 events, 6.4%). Of these nine bleeding events, eight were registered during hospital course. Six were access site related and two emerged due to pharyngeal injuries by the TEE probe. One of the two life-threatening bleedings, one was access site related while the other one occurred during follow-up and was intracranial hemorrhage. The patient with intracranial hemorrhage was not on oral anticoagulation. Ischemic events were less frequent. No stroke occurred. Only three TIAs were recorded; one during hospital stay and two during follow-up (Table III).

Correlation of primary and secondary endpoint

The incidence of HTPR to antiplatelet medication was similar in patients with- vs. without adverse clinical events. In patients with

| Table II. Primary endpoint (high on-treatment platelet reactivity). |
|-----------------|-----------------|-----------------|
| 140 PFO/ASD closures n=140 | Clopidogrel (VASP) | Aspirin (LTA) n=133* |
| Patients n (%) HTPR (PRI>50) | HTPR (Aggregation>20%) | LTPR (PRI<16) |
| 99 (70.7%) | 5 (3.8%) | 7 (5%) |

LTA = light transmission aggregometry; PRI = platelet reactivity index; VASP = vasodilator stimulated protein phosphorylation. *Seven patients were not administered aspirin because of aspirin intolerance.

| Table III. Secondary endpoint (adverse events). |
|-----------------|-----------------|-----------------|
| In-hospital | 6-Month follow-up | Overall |
| 30-Day mortality | 0 (0%) | 0 (0%) | 0 (0%) |
| Cardiovascular cause of death | 0 (0%) | 0 (0%) | 0 (0%) |
| Ischemic complications: | | | |
| Periprocedural myocardial infarction | 0 (0%) | 0 (0%) | 0 (0%) |
| Major stroke | 0 (0%) | 0 (0%) | 0 (0%) |
| Minor stroke | 0 (0%) | 0 (0%) | 0 (0%) |
| Transient ischemic attack | 1 (0.7%) | 2 (1.4%) | 3 (2.1%) |
| Occluder thrombus | 0 (0%) | 0 (0%) | 0 (0%) |
| Bleeding complications: | | | |
| Major bleeding | 1 (0.7%) | 0 (0%) | 1 (0.7%) |
| Minor bleeding | 6 (4.3%) | 0 (0%) | 6 (4.3%) |
| Major vascular complications | 0 (0%) | 0 (0%) | 0 (0%) |
| Minor vascular complications | 0 (0%) | 0 (0%) | 0 (0%) |

ischemic events no significant difference in their antiplatelet effects compared to patients without ischemic events could be detected. There was also no correlation between the incidence of bleeding events and either HTPR to clopidogrel or aspirin. Likewise no association between clopidogrel LTPR and the occurrence of bleeding was registered (Table IV).

Correlation of anticoagulation and secondary endpoint

Nineteen patients had permanent oral anticoagulation because of atrial fibrillation or previous pulmonary embolism. There was no significant higher incidence of bleeding complications in patients on oral anticoagulation compared to patients without additional oral anticoagulation (Table IVC).

Discussion

In this study the essential observations were: (i) HTPR to clopidogrel is very frequent in PFO/ASD closure patients; (ii) there were no ischemic/ thrombotic events, but three major bleeding events (two life threatening); (iii) there was no correlation between the incidence of clinical complications and HTPR to antiplatelet medication.

We detected HTPR to clopidogrel in 70.7% of our patients. Described in earlier studies is a wide range for insufficient antiplatelet effects between 5% and 44% in patients undergoing percutaneous coronary intervention. In several studies, various possible mechanisms for HTPR to clopidogrel have been reported. These include poor bioavailability, increased age, drug-drug interactions (especially statins, proton-pump inhibitors and calcium channel blockers), diabetes mellitus, high body mass...
index (BMI) and genetic factors [11, 12]. The patients in this study display only sparse concordance with the reasons for HTPR described above. The population in the present study is relatively young (54 ± 14 years) and healthy (only 5% are suffering from diabetes mellitus), therefore genetic factors could play a major role for impaired response to clopidogrel medication. Especially a loss of function polymorphism of the P450 enzyme CYP2C19 is found to be a relevant component in healthy young individuals [13]. Furthermore, analysis of clinical parameters and HTPR to antiplatelet medication in our study revealed male patients to have an increased incidence of HTPR to clopidogrel. (Supplemental 1). Conversely, different studies reported female gender to be a risk factor for clopidogrel [14, 15]. Yet, a meta-analysis in ~90 000 patients did not detect gender-based differences in response to antiplatelet medication [16]. Another reason for the high incidence of HTPR could be differences in dosing. It has been shown that loading doses of 600 mg result in lower rates of nonresponsiveness to clopidogrel (5–13%) in comparison to 300 mg (23–44%) [17].

Since we applied 600 mg in this study, the measured high resistant rate can thereby not be explained.

The prevalence of HTPR to aspirin is described with wide variations between 0 and 57%. Major factors for this variability are used dosage (≤100 mg vs. ≥300 mg; prevalence of 36% vs. 19%) and methods of measuring (LTA vs. point-of-care platelet function testing; 6 vs. 26%) [18]. In this study, we measured HTPR to aspirin by LTA and administered a daily dose of 100 mg aspirin. Still, we observed nonresponsiveness to aspirin in only 5 patients (3.8%). Genetic factors, incompliance, conditions that are associated with increased platelet turnover (diabetes mellitus, coronary artery disease, obesity) and drug-drug interactions (NSAIDs, metamizole) are correlated with high rates of HTPR to aspirin [19–21]. As the incidence of diseases like diabetes mellitus and coronary artery disease as well as analgesic comedication with NSAIDs or metamizole was low in our patients, the measured HTPR rate to aspirin of 3.8% seems reasonable.

HTPR to clopidogrel has been described to be associated with ischemic events and LTPR with bleeding events in patients with coronary artery disease [6]. In this study, we detected a very high rate of HTPR to clopidogrel in patients with PFO/ASD closure, but there was no association with the incidence of clinical complications. However, the incidence of adverse events was very low, therefore no reasonable evaluation can be made due to small numbers. Additionally, recent analyses suggest anti-inflammatory effects beside inhibition of platelet aggregation of clopidogrel contribute to its reduction of ischemic events in CAD patients beside its inhibition of platelet aggregation [22, 23]. However, as mentioned above, patients with interventional PFO/ASD closure are relatively young and healthy, therefore antiatherosclerotic effects of clopidogrel are unlikely to decrease the incidence of ischemic events.

No case of thrombus formation on the occluder was registered. Described is an occurrence of device adherent thrombi of 0–10% depending on the used device and antplatelet medication strategy [24]. We implanted the Amplatzer septal occluder (81% of the procedures) to the Figulla occlutech device (19% of the procedures). In accordance with our observations, no thrombus formation on these occluders was found in large scale investigations [25–27]. We monitored PFO/ASD closure patients carefully with transesophageal and transthoracic echocardiography and did not detect a thrombus formation at any time during procedure or follow-up examinations. At the moment, dual antplatelet medication with aspirin and clopidogrel is applied early after PFO/ASD closure, despite the lack of evidence. Looking at the reasons for this, Braun et al. described thrombus formation on the PFO Star device of first and second generation in 8 of 276 cases (2.9%). Subsequently, they added clopidogrel to the antplatelet regiment of aspirin alone and simultaneously improved the device resulting in no further detection of thrombus formation [28]. Since then, the application of aspirin and clopidogrel is widely used though there is no validated data, and therefore no definite recommendations. Especially, it is not known, whether the improvement of the closing device with internalization of the nitinol arms and consecutively no contact of these nitinol arms with the blood flow or the change of the antplatelet regimen led to the observed reduction in the incidence of thrombus formation on the occluder. Additionally, Krumdsorf et al. investigated thrombus formation in 1000 patients who underwent PFO/ASD closure and were treated with different antplatelet/anticoagulant regimes postinterventionally (warfarin alone, aspirin alone or dual antplatelet therapy with aspirin and clopidogrel). In 20 patients a thrombus formation could be detected, but a superiority of any of the regimes regarding the incidence of thrombus formation on the occluder was not observed [25].
Conclusion
With the data from the present study, we generate the hypothesis that additional antiplatelet medication with clopidogrel is questionable when using new generation SJM or Occlutech closing devices. We detected that HTPR to clopidogrel is very frequent (71% of patients) and despite that, no thrombus formation and no stroke or myocardial infarction occurred at all. On the other hand, three major bleedings including two life-threatening bleeding were observed (one intracranial hemorrhage). Further investigations in form of large-scale trials with clinical endpoints are required to reconfirm clopidogrel medication in PFO/ASD patients.

Study limitations
This was a hypothesis-generating pharmacodynamic study evaluating HTPR to clopidogrel and aspirin in PFO/ASD closure patients. Genetic polymorphisms involved in biotransformation of clopidogrel were not analyzed. It was an observational study with a heterogeneous patient population. However, it reflects a real world cohort. As we conducted a pilot study, it was not empowered to indicate significant differences in the subgroups regarding an association between complications and antiplatelet effects.

Ethics committee approval
The study conformed to the Declaration of Helsinki and was accepted by the University of Düsseldorf Ethics Committee.

Disclosures
The authors state, that there was no financial support, grant, contract or industrial sponsor.

Supplemental Material
Supplemental data for this article can be accessed on the publisher’s website.