# DOTTORATO DI RICERCA IN SCIENZE CLINICHE

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COORDINATORE Prof. Lorenzo Cosmi

# IMPROVING THE NET CLINICAL BENEFIT OF DUAL/TRIPLE ANTITHROMBOTIC THERAPY IN PATIENTS WITH ATRIAL FIBRILLATION AND ACUTE CORONARY SYNDROME: DISCOVERY AND VALIDATION OF PROGNOSTIC FACTORS FOR A TAILORED THERAPY

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**Dottorando**
Dott.ssa Martina Berteotti

**Tutore**
Prof.ssa Rossella Marcucci

**Coordinatore**
Prof. Lorenzo Cosmi

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# Improving the net clinical benefit of dual/triple antithrombotic therapy in patients with atrial fibrillation and acute coronary syndrome: discovery and validation of prognostic factors for a tailored therapy

**Background** Antithrombotic management of patients with atrial fibrillation (AF) undergoing percutaneous coronary intervention (PCI) can be challenging. Recent direct oral anticoagulant (NOAC) trials demonstrated the safety of double antithrombotic therapy (DAT) in comparison with triple antithrombotic therapy (TAT) in this setting (especially when a vitamin K antagonist -VKA- was part of it). However, this benefit is counterbalanced by a higher risk of ischaemic, mainly stent-related, events.

**Purpose** We sought to identify possible laboratory predictors of bleeding and ischaemic risk in a real-world population with concomitant AF and PCI.

**Methods** All consecutive patients with history of AF discharged from our cardiology ward with DAT or TAT after a PCI from April 2018 to March 2021 were enrolled in an observational registry. For all subjects, blood serum samples were collected and tester for D-Dimer, thrombin generation time, clot lysis time, platelet reactivity by arachidonic acid and ADP, erythrocyte aggregation and deformability, oxidative stress and DOAC concentration if appropriate. Major adverse cardiac and cerebrovascular events (MACCE) and major haemorrhagic or clinically relevant non major bleeding events together with therapeutic changes were recorded at 3 months follow-up for all patients.

**Results** A total of 147 patients were included in the present analysis (70.1% after acute coronary syndromes ACS and 29.9% after elective PCI); the mean age was 78±8 years and 48 (33%) were women. Ninety-one patients (62%) were discharged with TAT. Both in TAT and in DAT group DOACs were preferred (58% and 77%, respectively). In 93.4% of patients, clinicians chose clopidogrel as P2Y12 inhibitor. The median follow-up was 203 days (IQR 115-378). In this amount of time 25 patients experienced a MACCE (17%) and 23 patients a haemorrhagic event (15.6%), with no significant differences between TAT and DAT group. The incidence of all-cause death was 12.2% (18 patients); 11 subjects died from cardiovascular causes (7.4%). The independent predictors of MACCE at COX regression analysis were von Willebrand factor (HR 1.52, 95% C.I. 1.14 to 2.04, p=0.005) and P2Y12 dependent platelet reactivity (HR 1.56, 95% C.I. 1.00 to 2.42, p=0.049). In the first three months after discharge, when most MACCE occurred (56%), MACCE were associated with the presence of high platelet reactivity (HPR) to P2Y12 (MACCE free survival 95% vs. 81%, p=0.015) and dual HPR (both to P2Y12 and TXA2, MACCE free survival 92% vs. 71%, p=0.011). The only significant independent predictor of bleeding was low oxidative stress level, quantified by ORAC ratio (HR 7.77, 95% C.I. 1.61 to 37.38, p=0.011). In patients receiving Dabigatran (n=41) the cut off level of 101 ng/ml of plasmatic concentration discriminated patients at higher bleeding risk (96.0±3.9 vs. 35.7±19.8, p=0.001). Independent predictors of all-cause mortality were the following: age (HR 3.52, 95% C.I. 1.56 to 7.93, p=0.002), gender (HR for male sex 0.26, 95% C.I. 0.07 to 0.90, p=0.033), white cells blood count (HR 2.75, 95% C.I. 1.49 to 5.08, P=0.001) and red blood cells count (HR 0.41, 95% C.I. 0.22 to 0.76, p=0.005), and high oxidative stress, expressed as TBARS levels (HR 8.31, 95% C.I. 2.23 to 30.94, p=0.002).

**Conclusions** In a real-world unselected population, a significant incidence of ischemic as well as haemorrhagic events was observed both in patients on TAT and DAT early after discharge. Our data documented the relevance of the entity of platelet inhibition in patients with ACS suggesting that a laboratory evaluation of drug response might be useful in personalizing antithrombotic therapy in this setting. An unbalance in oxidative stress was associated with bleeding and death. Further studies are needed in order to confirm the association observed between low oxidative stress and bleeding.

Summary

# INTRODUCTION

## 1. Coronary artery disease and atrial fibrillation: epidemiology and prognosis

Atrial fibrillation (AF) is a global clinical problem affecting millions of people worldwide. According to systematic review of 184 studies, the estimated number of individuals with AF globally in 2010 was 33.5 million, 20.9 million men and 12.6 million women.[1] By 2030, 14 – 17 million AF patients are anticipated in the European Union, with 120 000 – 215 000 newly diagnosed patients per year.[2] The increase in AF prevalence can be attributed both to better detection of silent AF,[3] alongside increasing conditions predisposing to AF, such as hypertension, heart failure, coronary artery disease (CAD), obesity, diabetes or chronic kidney disease (CKD). [4–6] Above all, age is the most relevant risk factor: an octogenarian has a 9-fold higher risk of AF when compared to a subject of 50 years old.[7,8] AF is independently associated with a two-fold increased risk of all-cause mortality in women and a 1.5-fold increase in men.[9] AF is also associated with increased morbidity, such as heart failure and stroke.21,24,25 The annual incidence of ischemic stroke in AF patients is about 4,5%, 4-5 fold higher than that observed in general population and cardioembolic stroke is associated with particularly high risk of mortality, disability and prolonged hospitalization.[10]

On the other side, ischemic cardiomyopathy is the most common cardiomyopathy in high-income countries, and it is a leader cause of mortality and DALY (disability-adjusted life year), with more than 7,2 millions of deaths worldwide and 129 millions of annual DALY.[11,12] Data extrapolated from clinical trials, even with the relative bias, estimate an annual incidence of death of 1,2-2,4%, of cardiac death 0,6-1,4% d of non-fatal myocardial infarction (MI) up to 2.7%.[13] The incidence of MI is higher in men, and occurs later in women.[14,15] While STEMI incidence decreased appreciably in the last decade, the NSTEMI rate slightly increased.[16]

Among patients with AF, about 20% may have concomitant coronary artery disease.[17] On the other side, approximately 5–10% of patients undergoing PCI also AF.[18] These disease share association with some important cardiovascular risk factors. Besides, the presence of AF in patients with ACS negatively condition prognosis, increasing 30-days mortality by 2-times as compared to patients in sinus rhythm. In the ACACIA study (Acute Coronary Syndrome Prospective Audit) 3.393 ACS patients were enrolled; 4.4% of them had de-novo AF and 11.4% already had a diagnosis of AF. De novo AF was more common among STEMI patients, while preexisting AF was mostly observed in NSTE-SCA. After 1-year follow-up, de novo AF was associated with a worse long-term composite outcome of death, myocardial infarction and stroke (hazard ratio 1.66, p = 0.004). The odds ratio for the composite outcome was greatest for patients with new-onset AF with intermediate-risk NSTE-ACS (odds ratio 3.9, p = 0.02).[19]

## 2. Assessment of thromboembolic and hemorrhagic risk in AF

Thromboembolic risk in AF is not uniform and ranges from 0,4% to 12%/year, according to patient clinical profile, with a greater risk in patients with moderate-to-severe mitral stenosis and mechanical prosthetic

heart valve.[20] Interestingly patients without any risk factor have an annual thromboembolic risk lower than 1%.[21] The assessment of this risk is therefore of paramount importance. To this purpose guidelines support the use of $CHA_2DS_2$-VASc score (Figure 1) in all patients with AF except those with moderate-to-severe mitral stenosis and mechanical prosthetic heart valve, considered at very high risk.[22] Subjects with a score of 0 (or 1 for women) should not receive any antithrombotic treatment, while a score ≥ 2 for men and ≥ 3 for women, identify patients at high risk who must be treated with OAC (LOE IA).[23] In the intermediate range of 1 for men and 2 for women the recommendation is also in favor of OAC with a lower strength of evidence (IIa B).

| $CHA_2DS_2$-VASc risk factor | Points |
|---|---|
| **Congestive heart failure**<br>Signs/symptoms of heart failure or objective evidence of reduced left ventricular ejection fraction | +1 |
| **Hypertension**<br>Resting blood pressure >140/90 mmHg on at least two occasions or current antihypertensive treatment | +1 |
| **Age 75 years or older** | +2 |
| **Diabetes mellitus**<br>Fasting glucose >125 mg/dL (7 mmol/L) or treatment with oral hypoglycaemic agent and/or insulin | +1 |
| **Previous stroke, transient ischaemic attack, or thromboembolism** | +2 |
| **Vascular disease**<br>Previous myocardial infarction, peripheral artery disease, or aortic plaque | +1 |
| **Age 65–74 years** | +1 |
| **Sex category (female)** | +1 |

**Figure 1.** Clinical risk factors for thromboembolic events in the $CHA_2DS_2$-VASc score.[7]

On the other side, OAC is conditioned by an increase in hemorrhagic risk, with incidence of major bleeding ranging from 2 to 5%/year.[24] Several bleeding risk score have been developed, mainly in patient on VKA. One of the most adopted is HAS-BLED [hypertension, abnormal liver/renal function, abnormal renal/liver function (1 point each), stroke, bleeding history or predisposition, labile INR, elderly (.65 years), drugs/alcohol concomitantly (1 point each)].[25] A high bleeding risk score should generally not result in withholding OAC. Rather, bleeding risk factors should be identified and treatable factors corrected (figure 2).[7]

| Modifiable bleeding risk factors |
|---|
| Hypertension (especially when systolic blood pressure is >160 mmHg)[a,b,c] |
| Labile INR or time in therapeutic range <60%[a] in patients on vitamin K antagonists |
| Medication predisposing to bleeding, such as antiplatelet drugs and non-steroidal anti-inflammatory drugs[a,d] |
| Excess alcohol (≥8 drinks/week)[a,b] |
| **Potentially modifiable bleeding risk factors** |
| Anaemia[b,c,d] |
| Impaired renal function[a,b,c,d] |
| Impaired liver function[a,b] |
| Reduced platelet count or function[b] |
| **Non-modifiable bleeding risk factors** |
| Age[e] (>65 years)[a] (≥75 years)[b,c,d] |
| History of major bleeding[a,b,c,d] |
| Previous stroke[a,b] |
| Dialysis-dependent kidney disease or renal transplant[a,c] |
| Cirrhotic liver disease[a] |
| Malignancy[b] |
| Genetic factors[b] |
| **Biomarker-based bleeding risk factors** |
| High-sensitivity troponin[e] |
| Growth differentiation factor-15[e] |
| Serum creatinine/estimated CrCl[e] |

Figure 2. Modifiable and non-modifiable risk factors for bleeding in anticoagulated patients based on bleeding risk scores.[7]

# 3. Antithrombotic therapy for patients with AF

It is well-known oral anticoagulation (OAC) is the therapy of choice to reduce thromboembolic risk in AF patients. Vitamin K antagonists (VKA) proved better both than single and double antiplatelet therapy.[26,27] The ACTIVE study (which compared VKA with aspirin alone or plus clopidogrel) found a better efficacy in preventing cardioembolic risk with VKA, without differences in bleeding events.[28] Similar benefit was observed in another study comparing apixaban with aspirin in patients with unsuitable for VKA.[29]

Despite efficacy, VKAs have several limitations mainly due to unpreventable pharmacokinetic pharmacodynamic effects. In fact efficacy of VKA is optimal with time in therapeutic range (TTR)>70% acceptable for values di TTR between 61% and 70%.[30] Besides, VKA are associated with significant increase in major bleedings. The ISCOAT study found a 1.1% patient/year incidence of major bleeding, 0.25% of fatal bleeding and 0.44% of intracerebral hemorrhage.[31] Similar data were observed in the START registry.[32] VKAs are currently the only treatment with established safety in AF patients with rheumatic mitral valve disease and/or an artificial heart valve.

In four pivotal RCTs, direct oral anticoagulants (DOACs) apixaban, dabigatran, edoxaban, and rivaroxaban have shown non-inferiority to warfarin in the prevention of stroke/systemic embolism[33–36]. In a meta-analysis of these RCTs, DOACs were associated with a 19% significant stroke/systemic embolism risk reduction, a 51% reduction in hemorrhagic stroke, and a significant 10% reduction in all-cause mortality.

There was a non-significant 14% reduction in major bleeding risk, significant 52% reduction in ICH, and 25% increase in gastrointestinal bleeding with DOACs vs. warfarin.[37] Post-marketing observational data on the effectiveness and safety of DOACs vs. warfarin show general consistency with the respective RCT.[38–42] For Given this compelling evidence, DOACs are recommended as first line therapy in AF patients with a $CHA_2DS_2VASc$ score higher that 1 in men and 2 for women.[23]

## 4. Antiplatelet therapy after percutaneous coronary intervention

Even if the risks of late and (even more) very late stent thrombosis have declined considerably since the advent of newer- generation DESs, double antiplatelet therapy (DAPT) consisting of aspirin and a $P2Y_{12}$ receptor inhibitor represents the cornerstone of treatment in patients undergoing PCI.[43,44]
In the CURE study, DAPT with aspirin and clopidogrel was tested on about 12500 patients with NSTE- SCA who had undergone PCI or fibrinolysis, showing a 20% reduction in the combined endopoint (MI, stroke and cardiovascular death) as compared to aspirin.[45] This benefit was more consistent in patient treated with stenting.[46] Similar efficacy was observed in STEMI in COMMIT[47] and CLARITY trials.[48]

In patients with ACS undergoing PCI, more potent oral $P2Y_{12}$ antagonists, such as prasugrel and ticagrelor, have been shown to further reduce the relative risk of adverse cardiovascular events by approximately 15–20% compared with clopidogrel but at the expense of an increased bleeding risk.[49,50] For such reason prasugrel and ticagrelor are recommended in patients with ACS.[51] Instead clopidogrel should be the default treatment after elective PCI, or in ACS subjects with a contraindication for other $P2Y_{12}$ inhibitors.[52,53]

Several studies confirmed that platelet inhibition in response to clopidogrel in heterogeneous, with the presence of pharmacologic resistance in about 20-30% of subjects.[54] Also for this reason, the prescription of ticagrelor and prasugrel is contemplated after elective PCI in selected cases (i.e. high risk of stent thrombosis), even if never evaluated in RCT.[53]

After elective PCI DAPT is generally recommended for 6 months,[53] while in ACS, DAPT should be prescribed for 12 months, irrespectively from the acute treatment strategy.[52,55] In cases of bleeding risk prevailing over ischemic one, DAPT can be limited to 1 to 3 months in elective PCI and 6 months after ACS.[53,56] To assess bleeding risk in this contest, the PRECISE-DAPT has been proposed by 2017 ESC guidelines on DAP.[57]

On the other side, DAPT and PEGASUS evaluated DAPT prosecution beyond 12 months with clopidogrel/prasugrel and ticagrelor, respectively, showing a reduction in ischemic events at the price of increased bleedings.[58,59] In this case, the DAPT score has been validated. Within the DAPT trial, a score ≥ 2 selected patients who showed a reduction in MI/stent thrombosis and cardiovascular or cerebrovascular events risk [number needed to treat (NNT) for benefit for ischemic event reduction = 34] after a prolonged, 30-month DAPT, with only a modest increase in bleeding risk (NNT for harm = 272).[60]

# 5. Antithrombotic therapy in patients with atrial fibrillation undergoing PCI

The optimal antithrombotic treatment regimen for patients with AF undergoing PCI is a clinical conundrum. Although dual antiplatelet therapy with aspirin and a $P2Y_{12}$ inhibitor is effective in reducing cardiac events and stent thrombosis after PCI (STAR trial),[61,62] oral anticoagulation (OAC) is the therapy of choice for the prevention of stroke and systemic embolism in patients with AF (ACTIVE-W trial).[28]

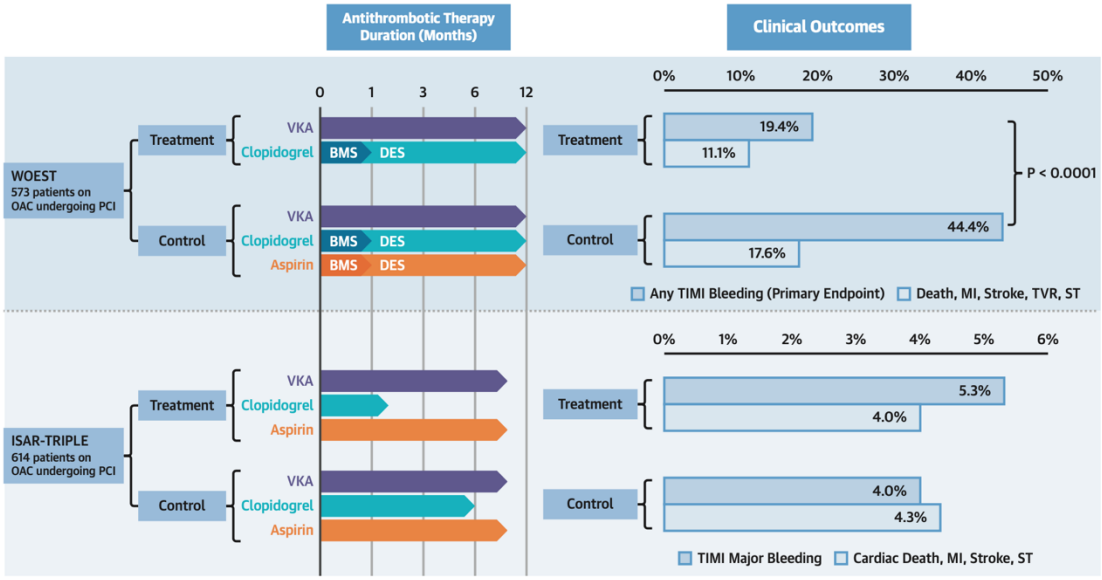
However, triple therapy is known to be associated with an excess in major bleeding, ranging from 4% to 12% within the first year of treatment,[63,64] and haemorrhagic risk is 4 times higher than that observed with aspirin alone.[65] The occurrence of a bleeding complication is known to affect the prognosis of the patients, as it determines the discontinuation of all antithrombotic drugs.[66]

## 5.1 Triple or double antithrombotic therapy in VKA era

Before the introduction of DOAC in clinical practice, triple antithrombotic therapy (TAT) was performed with warfarin, aspirin and clopidogrel. However considering the high bleeding risk associated with this therapy,[67] the identification of an alternative more sustainable regimen has been searched for years.

Two RCT explored this topic. In 2013 WOEST (What is the Optimal antiplatElet and anticoagulant therapy in patients with oral anticoagulation and coronary StenTing) trial randomized 573 patients with an indication to OAC who underwent PCI to TAT (warfarin, aspirin, clopidogrel) or double therapy (DAT) with warfarin and clopidogrel.[68] At 1-year follow-up, DTA showed superior safety (64% reduction in hemorrhagic events- primary endpoint), but also superior efficacy (40% reduction in the combined incidence of stroke, MI, revascularization, stent thrombosis, death). However, the sample size was not adequate to establish a clear efficacy benefit and the low proportion of ACS subjects (27.5%) reduce generalizability. Besides, thi benefits on ischemic events is mostly attributable to significative reduction in non-cardiac mortality. Since there are no plausible pathophysiologic mechanism able to explain tis gain, casualty could not be excluded. Another limitation of WOEST study was the definition of primary endpoint (any bleeding event according to TIMI, GUSTO or BARC classification: the incidence of these events was extremely high (19.4 vs 44.4%), but mostly attributable to minor bleeding, and the major difference between TAT and DAT was observed with respect of cutaneous bleeding (10.6 vs 2.5%), while incidence of ICH was the same (1.1%). Nevertheless, this study open the way to the research of other theraputical strategies.

In 2015 ISAR-TRIPLE (Triple Therapy in Patients on Oral Anticoagulation After Drug Eluting Stent Implantation) trial was published.[69] It enrolled 614 patients who underwent PCI with DES; of them 32% had ACS. These subjects were randomized into 2 groups treated with VKA, aspirin 75-200 mg/die and clopidogrel 75mg/die for 6 weeks or 6 months. The primary endpoint was composed by death, MI, stent thrombosis, stroke or major bleeding. After 9 months the primary endpoint was comparable (9.8% shorter TAT vs 8,8% longer TAT). The main limitation of this trial was, as for WOEST, the scarcity of ACS patients. This trial suggest that a modulation in TAT duration could be a possible way to reduce bleeding risk.[70]




BMS = bare-metal stent; DES = drug-eluting stent; ISAR-TRIPLE = Triple Therapy in Patients on Oral Anticoagulation After Drug Eluting Stent Implantation; MI = myocardial infarction; OAC = oral anticoagulation; PCI = percutaneous coronary intervention; ST = stent thrombosis; TIMI = Thrombolysis In Myocardial Infarction; WOEST = What is the Optimal antiplatElet & Anticoagulant Therapy in Patients With Oral Anticoagulation and Coronary StenTing.

**Figure 3.** Study Design and Key Outcomes of Double Therapy in OAC Patients Undergoing PCI in the Era of Vitamin K Antagonists: WOEST and ISAR-TRIPLE (from Capodanno D., et al[71]).

## 5.2 Triple or double therapy with DOAC

RE-LY was the only trial comparing DOAC with VKA in AF in which concomitant DAPT was allowed (about 800 subjects). In those patients in TAT a higher bleeding risk was observed, especially with dabigatran 150mg and warfarin.[33]

More recently, 4 trial specifically comparing DAT with TAT with DOAC or VKA were published.

The first one was PIONEER AF-PCI (Open-Label, Randomized, Controlled, Multicenter Study Exploring Two Treatment Strategies of Rivaroxaban and a Dose-Adjusted Oral Vitamin K Antagonist Treatment Strategy in Subjects with Atrial Fibrillation who Undergo Percutaneous Coronary Intervention) trial.[72] It randomized 2,214 patients with nonvalvular AF who had just undergone PCI with stent placement to one of three treatment groups:

- Rivaroxaban 10-15 mg daily+$P2Y_{12}$ inhibitor x12 months (WOEST-like strategy);
- Rivaroxaban 2.5 mg BID+DAPT x1, 6, or 12 months (ATLAS ACS-2-like strategy);[73]
- Warfarin+DAPT x1, 6, or 12 months.

At 12 months, the primary outcome of clinically significant bleeding was lower in the rivaroxaban groups than the warfarin groups (16.8% vs. 18.0% vs. 26.7%). There was no significant difference in the rate of major adverse cardiovascular events (CV mortality, MI, or stroke) between the three groups. However, this trial was not powered to detect a difference between the groups.

The 2017 RE-DUAL PCI (Randomized Evaluation of Dual Antithrombotic Therapy with Dabigatran versus Triple Therapy with Warfarin in Patients with Nonvalvular Atrial Fibrillation Undergoing Percutaneous Coronary Intervention) trial[74] randomized 2725 patients with AF undergoing PCI (for acute coronary syndrome in 51% of cases) to

- TAT using aspirin, thienopyridine (plavix or ticagrelor), and warfarin (goal INR 2-3)
- DAT using thienopyridine and dabigatran 150mg twice daily
- DAT using thienopyridine and dabigatran 110mg twice daily

The primary outcome was the incidence of major or clinically relevant non-major bleeding. Patients receiving triple therapy were given aspirin for 1-3 months post-PCI and all patients received a thienopyridine for 12 months post-PCI. At mean follow-up 14 months, TAT was associated with an 11.5% absolute increase in the primary outcome over the dabigatran 110mg twice daily DAT group and a 5.5% absolute increase over the dabigatran 150mg twice daily DAT group. In secondary efficacy analyses assessing for thrombotic events, rates of MI, stroke, systemic embolism, death, or unplanned revascularization were similar across the three groups, although a pooled analysis combining both doses of dabigatran dual therapy demonstrated a modest and non-significant 1.1% absolute increase in thrombotic events or death compared to the TAT group. Indeed, the incidence of stent thrombosis was nearly doubled in DAT group with dabigatran 110mg twice daily as compared to TAT group (1.5% vs. 0.8%).

Subsequent pot-hoc analysis did not show any difference in the primary endpoint between groups, apart from the subpopulation of patients older than 80 years old, in which bleedings were reduced with 110mg.[75]

The trials mentioned so far, compared two different strategies (DAT vs. TAT), rather than the type of anticoagulant agent (DOAC vs. VKA).

This topic was better addressed by AUGUSTUS trial,[76] that randomized 4614 patients with known AF presenting with ACS (61%) and/or undergoing elective PCI (39%) in a 2x2 factorial design to

- apixaban versus warfarin,
- aspirin versus placebo

At 6 months, apixaban was associated with a 4.2% absolute reduction in major or clinically relevant non-major bleeding when compared to warfarin. Apixaban was also associated with a 3.9% absolute reduction in death or hospitalization when compared to warfarin (driven primarily by reduction in hospitalization). Aspirin use in addition to oral anticoagulation was associated with a 7.1% absolute increase in major or clinically relevant nonmajor bleeding. Thrombotic events were similar across individuals randomized to anticoagulation with apixaban versus warfarin, and those randomized to aspirin versus placebo. Notably, however, there was a nonsignificant 0.4% absolute numerical increase in definite/probable stent thrombosis in patients not receiving aspirin. It should be noticed that mean time of enrollment after PCI was 6 days, meaning that the DOAC-DAT strategy was initiated after a short length of DOAC-TAT around the index event.

The last published trial was ENTRUST-AF PCI (EdoxabaN TReatment VersUS Vitamin K Antagonist in PaTients With Atrial Fibrillation Undergoing Percutaneous Coronary Intervention).[77] This trial compared 12 months edoxaban 60mg + a $P2Y_{12}$ inhibitor, with VKA+ a $P2Y_{12}$ inhibitor and aspirin for 1 to 12 months. The study population included 1.506 patients with AF who had undergone stenting for ACS (51.6%) or stable CAD (48.4%). The primary endpoint (major or clinically relevant non-major bleeding) occurred in 128 patients (17.0%) in edoxaban group and in 152 patients (20.1%) in VKA group (HR: 0,83, IC 95%: 0,654-1,047, p=0,001 for non-inferiority). ICH occurred in 4 subjects (0.58%) treated with edoxaban and 9 (1.32%) with VKA. The primary efficacy endpoint (cardiovascular death, stroke, systemic embolic events, MI and stent thrombosis) occurred in 49 patients (6.5%) in the edoxaban group and in 46 (6.1%) in the VKA group (HR 1.06; 95% CI 0.71–1.59). However, also this trial was not powered enough to detect any statistically significant difference in efficacy endpoint.

A meta-analysis of the four trials encompassing 10 234 patients (DAT = 5496 vs. TAT = 4738) demonstrated that the primary safety endpoint (ISTH major or clinically relevant non-major bleeding) was significantly lower with DAT compared with TAT [risk ratio (RR) 0.66, 95% confidence interval (CI) 0.56–0.78; P < 0.0001; I2 = 69%]. However, this benefit was counterbalanced by a significant increase of stent thrombosis (RR 1.59, 95% CI 1.01–2.50; P = 0.04; I2 = 0%) and a trend towards higher risk of MI with DAT. There were no significant differences in all-cause and cardiovascular death, stroke and major adverse cardiovascular events. The comparison of NOAC-based DAT vs. VKA-TAT yielded consistent results and a significant reduction of intracranial hemorrhage (RR 0.33, 95% CI 0.17–0.65; P = 0.001; I2 = 0%).[78]

Other data will be provided by ongoing trials AVIATOR-2, COACH-AF-PCI e WOEST 2.[64]

**Table 1.** RCTs assessing DAT including a DOAC versus TAT including VKA in patients with atrial fibrillation undergoing PCI (from De Rosa S., et al).[79]

| Trial name | PIONEER AF-PCI | RE-DUAL PCI | AUGUSTUS | ENTRUST AF-PCI |
|---|---|---|---|---|
| **Trial type** | Randomized, open label | Randomized, open label | Randomized, open label | Randomized, open label |
| **Study enrollment** | May 2013-July 2015 | July 2014-May 2017 | Sep 2015-April 2018 | Feb 2017-May 2018 |
| **Major inclusion criteria** | Age ≥ 18 years with non-valvular AF within last 1 year and who had just undergone PCI with stent placement (bare metal or drug eluting) for stable angina or acute coronary syndrome | Age ≥ 18 years with non-valvular AF within last 1 year and who had just undergone PCI with stent placement for stable angina or acute coronary syndrome | Age ≥ 18 years with previous, persistent, permanent or paroxysmal non-valvular AF; recent acute coronary syndrome or PCI, planned use of P2Y12 inhibitor for at least 6 months | Age ≥ 18 years, atrial fibrillation requiring oral anticoagulation, successful PCI for stable CAD or ACS |
| **Major exclusion criteria** | History of stroke/TIA, significant gastrointestinal bleeding within 12 months before randomization, eGFR<30 ml/min, Hgb<10 g/dl | Mechanical or biological heart valves, cardiogenic shock, prior stroke, surgery, gastrointestinal bleeding, major bleeding within 1 month prior to randomization, Hgb<10 g/dl, eGFR<30 ml/min, active liver disease | History of intracranial haemorrhage, recent or planned CABG, coagulopathy, ongoing bleeding, contraindication to either VKA, apixaban, P2Y12 inhibitors or aspirin; severe renal insufficiency | Mechanical heart valves, moderate-to-severe mitral stenosis, end-stage renal disease, other major comorbidities |
| **Treatment arm** | Rivaroxaban + single antiplatelet therapy with clopidogrel/ prasugrel/ticagrelor for 12 months duration | Dabigatran 110 mg twice daily or 150 mg twice daily + clopidogrel/ ticagrelor | Two-by-two factorial design:<br>• Apixaban vs VKA (target INR = 2.0–3.0);<br>• ASA 81 mg/die vs placebo | Edoxaban 60 mg once daily + clopidogrel 75 mg for 12 months by default: alternatively prasugrel 5/10 mg once daily or ticagrelor 90 mg twice daily at the investigator's discretion |
| **Control arm** | ASA + clopidogrel/prasugrel/ ticagrelor + warfarin, duration 1, 6, or 12 months pre-specified per treating physician | Aspirin + clopidogrel/ ticagrelor + warfarin | See cell above for randomized treatments.<br>In addition clopidogrel/prasugrel/ ticagrelor for 6 months | VKA + clopidogrel 75 mg for 12 months by default: alternatively prasugrel 5/10 mg once daily or ticagrelor 90 mg twice daily at the investigator's discretion + ASA 100 daily for 1–12 months |
| **Follow up, months (mean)** | 12 months | 14months | 6 months | 12 months |
| **Primary outcome (primary safety endpoint)** | Clinically significant bleeding (a composite of TIMI major and minor bleeding, or bleeding requiring medical attention) | ISTH major bleeding or clinically relevant non-major bleeding event | ISTH major or clinically relevant nonmajor bleeding | Composite of major or clinically relevant non-major (CRNM) bleeding (according ISTH) |
| **Secondary outcome (secondary efficacy endpoint)** | MACE (a composite of death, MI or stroke), each component of MACE and stent thrombosis | Composite of death, MI, stroke, systemic embolism or unplanned revascularization | A composite of death or hospitalization; a composite of death or ischemic events; each component of the composite endpoints; Acute Myocardial Infarction, Urgent revascularization and stent thrombosis | Stroke, ischemic stroke, haemorrhagic stroke, systemic embolic events, MI, all-cause death, cardiovascular or unexplained death |

https://doi.org/10.1371/journal.pone.0235511.t001

## 5.3 Guidelines recommendations

Multiple guidelines and consensus documents have been published over the past decade to inform clinicians on the optimal antithrombotic strategy for AF patients undergoing PCI.[71]

As far as the ***type of OAC*** drug is concerned, ACC/AHA/HRS and ESC guidelines[23,80,81] agree that, in the absence of contraindications, DOACs should be preferred to VKAs due to the lower risk of bleeding previously demonstrated to be a class effect.[37] In patients with an indication for a VKA (moderate to severe mitral stenosis and mechanical prosthetic heart valves and end-stage renal disease), the VKA dosing should be carefully regulated with a target INR of 2.0 - 2.5 and TTR>70%. In TAT regimens, both documents recommend using approved DOAC doses proven to be effective in regulatory trials of AF, with dose reductions as per the respective package labels. In the case of dabigatran and rivaroxaban, the lower dosage of 110mg bid and 15 mg respectively is suggested in patient at high bleeding risk (since these two regimens were tested in the relative RCT). After discontinuation of the $P2Y_{12}$ inhibitor, OAC should be continued at full stroke prevention doses.

For what concerns ***TAT duration***, recommendations have changed after the publications of all TAT vs. DAT RCTs. Before AUGUSTUS trial, TAT was considered from very short (e.g., until after successful PCI) to

extended (e.g., 6 months) depending on various clinical scenarios.[51] Based on the North American expert consensus document, the default approach is DAT, and thus to keep aspirin only in the periprocedural period and during hospital stay,[81] while the European guidelines recommend DAT as an alternative to TAT only to patients at baseline high bleeding risk.[51] On the other side, the North American document indicate that TAT for up to 1 month can be considered in patients who have high thrombotic risk and low bleeding risk.[81] In contrast, the European consensus document recommend 1 month of TAT as a default approach, or longer-term (3 to 6 months) TAT if the thrombotic risk exceeds the bleeding risk [51] (figure 4).

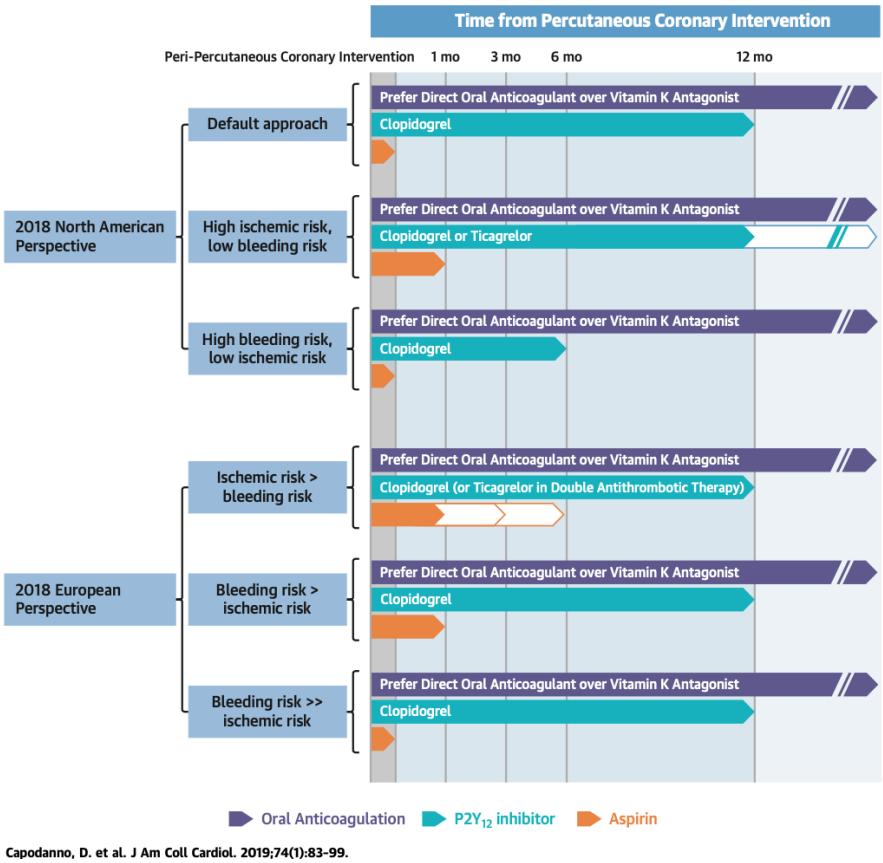



**Figure 4.** Consensus Recommendations on the Practical Management of Oral Anticoagulation and Antiplatelet Therapy in Patients With Atrial Fibrillation Undergoing Percutaneous Coronary Intervention, from Capodanno D. et al.[71]

After the publication of the latest trials on this topic, guidelines recommendation changed. Updated guidelines and expert consensus documents in Europe and the United States are now relatively aligned.[23,80] In fact both recommendations agree that aspirin should be maintained for 1 to 7 days (or to hospital discharge), with the option to prolong up to 30 days if the thrombotic or ischemic risk is high and the bleeding risk is low (figure5). Some characteristics to be evaluated when estimating patient risk are listed in figure 6.[23]

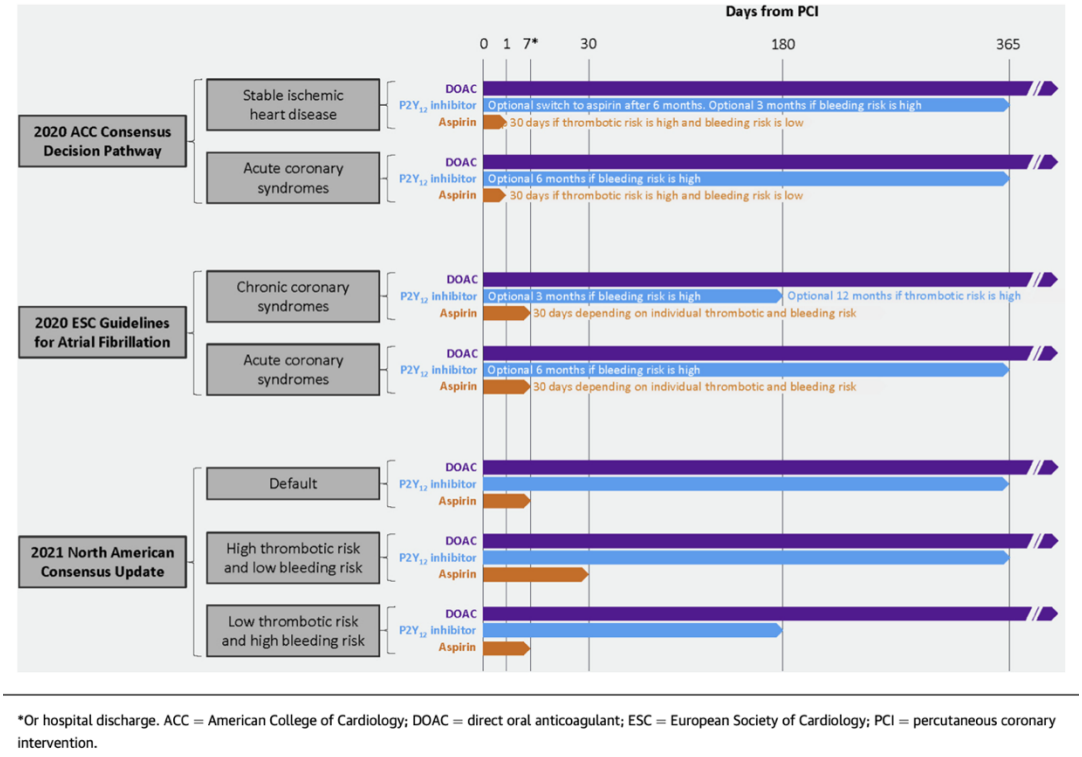



**Figure 5.** Management of Antiplatelet Therapy in Patients With Atrial Fibrillation Undergoing PCI Treated With DOACs According to Relevant Consensus Documents and Guidelines Since 2020. From Capodanno D.[82]

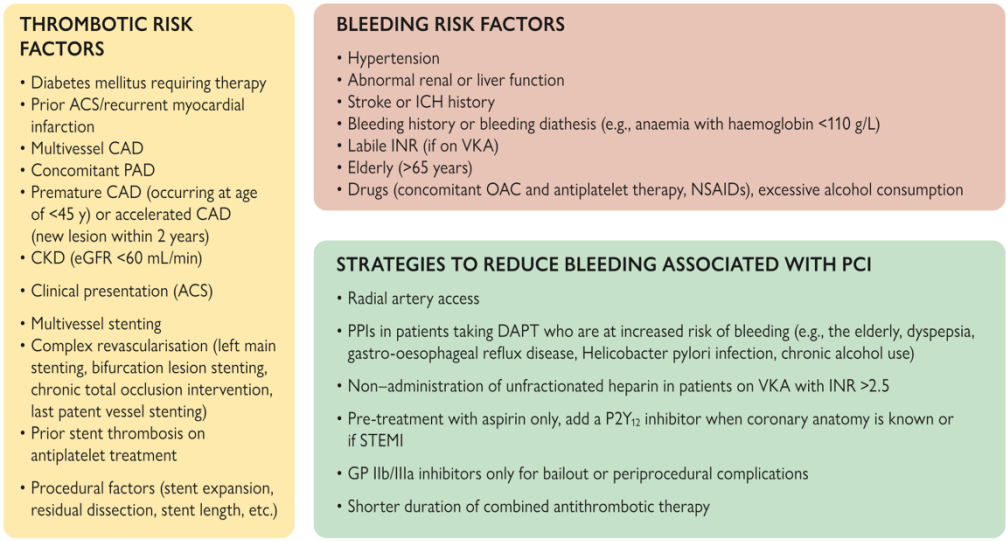



**Figure 6.** Thrombotic and bleeding risk factors that should be considered in patients who are candidate to TAT.[23]

Another important topic is to choose a specific ***antiplatelet drug to be discontinued*** in the transition from TAT to DAT. Both guidelines states that a $P2Y_{12}$ inhibitor should be used without aspirin. This recommendation is based on the well-established notion that, post-PCI, the use of a $P2Y_{12}$ inhibitor is pivotal for the prevention of thrombotic complications, despite the substantial variability in the platelet

response to clopidogrel.[83] However, experience with ticagrelor or prasugrel is minimal, with most data comes from RE-DUAL PCI trial, where 12% of patients received ticagrelor with dabigatran. Although no statistical interaction was noted between the treatment effect of the 2 tested doses of dabigatran and the use of clopidogrel or ticagrelor for DAT, the absolute rates of bleeding were higher when ticagrelor was used in TAT combinations as compared with DAT.[74,84] For this reason, ticagrelor and prasugrel are contraindicated as part of TAT, while 2019 ESC guidelines on chronic coronary syndromes suggested that dual therapy with an OAC and either ticagrelor or prasugrel may be considered in patients with a moderate or high risk of stent thrombosis, irrespective of the type of stent used.[53]

The $P2Y_{12}$ inhibitor should then be maintained for 12 months (or 6 months in patients at lower risk for thrombotic or ischemic complications and/or those at higher risk for bleeding).

At 12 months from PCI, ideally, the patients should continue on OAC alone, based on registry and other observational data showing that in patients with stable CAD, the addition of antiplatelet therapy to OAC increases bleeding without adding ischemic protection compared with OAC alone.[85–87] The OAC- ALONE (Optimizing Antithrombotic Care in Patients With AtriaL fibrillatiON and Coronary stEnt) study, prematurely terminated after enrolling 696 patients in 38 months, did not establish non-inferiority of OAC alone to combined OAC and SAPT in patients with AF and stable CAD beyond 1 year after PCI. However, because patient enrollment was prematurely terminated, this study should be considered inconclusive. On the same subject, the AFIRE (Atrial Fibrillation and Ischemic Events With Rivaroxaban in Patients With Stable Coronary Artery Disease Study) study randomly assigned 2236 patients with AF who had undergone PCI or CABG more than 1 year earlier or who had angiographically confirmed coronary artery disease not requiring revascularization to receive monotherapy with rivaroxaban or combination therapy with rivaroxaban plus a single antiplatelet agent. The trial was stopped early because of increased mortality in the combination-therapy group. Rivaroxaban monotherapy was noninferior to combination therapy for the primary efficacy end point (composite of stroke, systemic embolism, MI, unstable angina requiring revascularization, or death from any cause) and superior to combination therapy for the primary safety end point (major bleeding). Therefore, OAC monotherapy should be preferred in most patients after 12 months, unless concerns of thrombotic risk prevail, suggesting the need for continued DAT on a case-by-case basis.

# METHODS

## 1. Study population

The study population was enrolled in the observational prospective "Registry on the prescription of anticoagulant agents in association with double antiplatelet therapy in high cardiovascular risk patients" (number of registry of ethics committee's opinion: 12485_bio).

The study started on April 2018. In the present analysis we included AF patients with indication to anticoagulant therapy who underwent PCI from April 2018 to March 2021 in the Cardiovascular department of the Careggi University hospital, Florence (Structural Interventional Cardiology-Director Prof. Carlo Di Mario; General Cardiology-Director Prof. Niccolò Marchionni; Interventional cardiology-Director Prof. Niccolò Marchionni). Patients with a life expectancy lower than one year were excluded.

## 2. Enrollment

Patients with the required characteristics, were informed about the study procedures and then sign the informed consent. Clinical data inserted in the registry were the following: sex, date of birth, age, weight (kg), height (cm), prevalence of traditional cardiovascular risk factors and known atherosclerotic disease, previous history of MI, PCI or CABG, left ventricular ejection fraction (LVEF, %), white blood cells count [WBC/mL], red blood cells count [RBC, 106/mL], Hemoglobin [Hb, g/dL], Hematocrit [Htc, %], platelets [PLT, /mL], mean corpuscular volume [MCV, fl], creatinine [mg/dL].

Information about index event were also collected such as CAD presentation (stable, UA, NSTEMI, STEMI) and procedural aspects (number of diseased and treated vessels, total stent length and type of stent implanted).

For each patient $CHA_2DS_2$-VASc and HAS-BLED scores were calculated, and therapy at discharge was registered, including type of antiplatelet agents (aspirin, clopidogrel, ticagrelor or prasugrel) anticoagulant (VKA or DOAC), statins and PPI.

## 3. Laboratory analyses

Blood samples were obtained for all subjects and the following analysis were performed: platelet function tests (ADPtest in citrate [U], ASPItest in citrate [U], TXA2 dependent platelet reactivity [ARU], $P2Y_{12}$ dependent platelet reactivity [PRU]); redox balance - ORAC (oxygen radical absorbance capacity), TBARS (Thiobarbituric acid reactive substances); coagulation parameters (prothrombin time, expressed in sec, activity [%], ratio and INR, activated partial thromboplastin time [sec, aPTT], thrombin generation expressed as ETP [nM x min], peak [nM]), erythrocyte aggregation index [%], elongation index, D-D XDP [ng/mL FEU], clot lysis time [min], plasmatic DOAC concentration [ng/mL].

Blood was collected in tubes with anticoagulant (1.8 mg/ml EDTA), as well as in tubes without anticoagulant. Tubes were centrifuged at room temperature at 1500 × g for 15 min, and the supernatants (plasma/serum) will be stored in aliquots at −80° until measurements of biomarkers.

## 3.1 Platelet functional tests

### Multiplate analyzer

Whole blood aggregation was assessed in whole blood by the Multiplate analyzer (Roche Diagnostic). This instrument can perform up to 5 parallel aggregometry measurements assessing the change in impedance caused by the adhesion of platelets onto sensor units formed by silver-covered electrodes.[88] Multiplate aggregation was performed in citrated anticoagulated whole blood by using as agonists: ADP, 10 µmol/L, final concentration; AA, 1mmol/L, final concentration; and collagen, 2 µg/mL, final concentration.

### VerifyNow System

The VerifyNow System is a turbidimetric based optical detection system utilizing microbeads and which measures platelet induced aggregation as an increase in light transmittance.

#### 1. $P2Y_{12}$ Assay System

The VerifyNow-$P2Y_{12}$ Assay/VerifyNow PRU Test is a rapid test that uses ADP to stimulate platelets in the presence of PGE1 [Prostaglandin E1] and which inhibits activation downstream of a second ADP receptor $P2Y_1$ - making the assay more sensitive and specific for the activity of the $P2Y_{12}$ receptor and of drugs that bind to the P2Y12 receptor. The patient citrated whole blood sample is introduced into the assay system, which consists of Fibrinogen-coated microbeads and the platelets are activated by the inclusion of ADP. Activation of platelets via the ADP [20 µmol] in the device activates the GPIIb-IIIa receptor and this then binds to the Fibrinogen-coated microbeads. The aggregation of the beads leads to a change in OD which is measured. The VerifyNow $P2Y_{12}$ assay reports results in $P2Y_{12}$ Reaction Units (PRU). The cut-off value used for identification of patients with ACS, undergoing percutaneous coronary intervention at higher risk of adverse cardiovascular events is 208 PRU.[89]

#### 2. VerifyNow Aspirin Assay

The methodology is very similar to the VerifyNow-$P2Y_{12}$ Assay/VerifyNow PRU Test but arachidonic acid is incorporated to measure the response of the platelet to aspirin. In an individual on aspirin, aspirin irreversibly inhibits the COX-1, the enzyme that converts arachidonic acid to Thromboxane A [TxA2] and which ultimately activates the GPIIb/IIIa receptor involved in platelet aggregation. In presence of aspirin the aggregation does not occur. The VerifyNow Aspirin Assay reports results in Aspirin Reaction Units (ARU) and it measures the response of the platelet to Aspirin. Cut-off value is 550 ARU.[90]

## 3.2 Hemorheology parameters

### Red Blood Cells Deformability

Red blood cell deformability, expressed as the Elongation Index (EI), is presented in a deformability curve. A thin layer of RBC's, suspended in PVP, is sheared between two concentric cylinders. A known shear stress is applied to the cells, resulting in the elongation (deformation) of the RBC's. This process is measured by a laser beam diffraction pattern; captured with a video camera and analyzed by a computer.

When deforming under increasing shear stress, RBCs change gradually from thee biconcave towards a prolate ellipsoid morphology and orient themselves along the flow vector in the gap, i.e., tangential to the axis. Red blood-cell analyzer (LORCA) by a transition from a circular into an elliptic diffraction pattern (Figure 7) which is oriented perpendicular to the orientation of the elongated cells.

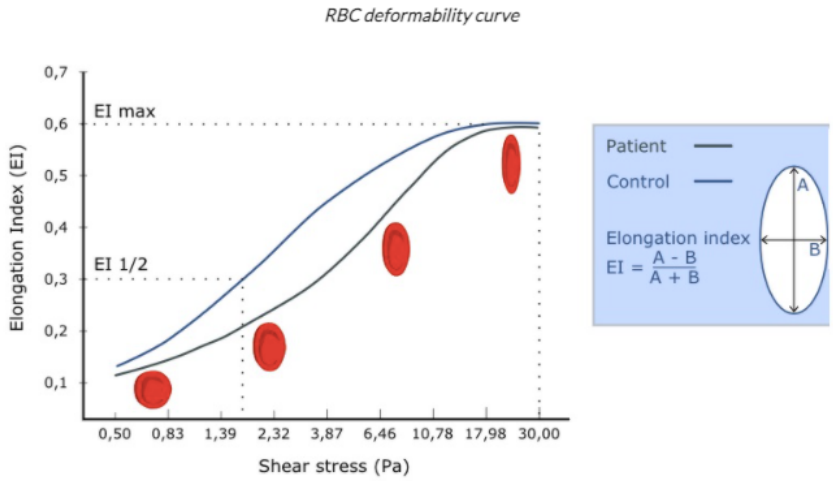



**Figure 7.** RBC deformability curve.

The Elongation Index {EI) is used as a measure of RBC deformability. It is calculated from an isointensity curve in the diffraction pattern using ellipse fitting and is defined as (A-B)/(A+B), where A and B are the vertical and horizontal axes of the ellipse, respectively. The EI is determined a user specified number of times at each shear stress and the mean value is plotted versus the corresponding shear stress (Pa) on the computer screen. Increase of shear stress in logarithmic steps from 0.3 to 53 Pa results initially in a rapid increase of EI until ~ 10 Pa, followed by a slower increment (see Figure 7). For comparison of various blood samples, the near-maximum value of EI at 30 Pa was chosen. The EI for normal cells at rest is zero and may increase to 0.6 at 300 Pa. The instrument is calibrated with 5 um diameter polymer microspheres {EI{EI = 0), while an additional calibration can be done with a standard ellipse pattern put on the screen, having a major (A) and minor (B) axis such that {A-B)I{A+B){A-B)I{A+B) is 0.

### RBC aggregation

Erythrocyte aggregation was also measured by LORCA. The measurement is based on the detection of laser back-scattering from the sheared (disaggregated), then unsheared (aggregating) blood, performed in a

computer-assisted system at 37 °C. Back-scattering data are evaluated by the computer and aggregation index (AI), amplitude of the aggregation (AMP), which is the total extent of aggregation, aggregation half time (t1/2) are calculated on the basis that there is less light back-scattered from aggregating red cells. Aggregation measurements were determined using RBCs in autologous plasma adjusted to 40 per cent haematocrit (Hct). Blood was fully oxygenated before measurements.

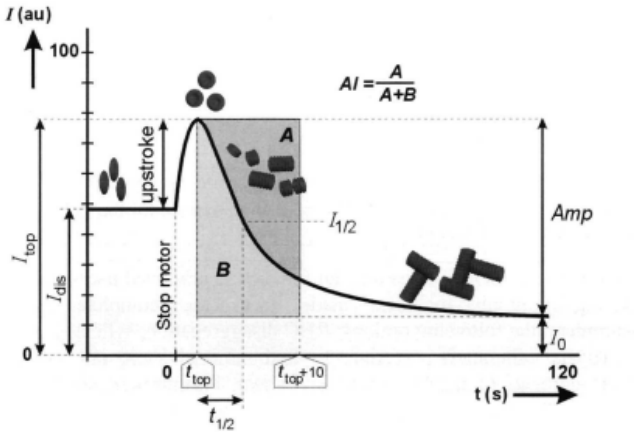



Figure 8. Syllectogram og RBC aggregation.

After a short period of rotation at an adjustable shear rate, high enough to cause complete disaggregation, the motor of LORCA instrument is stopped abruptly and the elongated and orientated RBC retake their normal, biconcave, shape and orientate randomly. The latter causes an increase in scatter intensity (upstroke) monitored by the photodiode sensor. This is followed by the aggregation process, reflected by a decrease in laser backscatter intensity (Isc). Initially, mainly 2D rouleaux are formed followed by the slower formation of 3D networks. The displayed curve, obtained by plotting Isc versus time (Figure 8), is called syllectogram. Light backscatter intensities are expressed in arbitrary units (au). Calibration is performed with water (zero reflection) and a special calibration fluid, as described in the technical reference of the instrument. Various indices, reflecting total extent, kinetics, avidity and pattern of aggregation, are calculated as indicated in the syllectogram (Figure 8), having a logarithmic time scale. Both the aggregation measuring procedure and the subsequent analysis of the syllectogram are computer controlled. The total extent of aggregation is shown by the amplitude (AMP), while the kinetic aspect is represented by the aggregation half time (t1/2). In order to combine this static and kinetic parameter, an index (AI) reflecting a relevant part of the syllectogram, arbitrarily taken from the top to 10 s thereafter, was calculated.

## 3.3 Thrombin generation

Venous blood samples anticoagulated with 0.129 M sodium citrate (ratio 9:1) and without anticoagulant were be taken from each patient. Citrated and serum samples were centrifuged at room temperature (1500 × g) for 15 min. The supernatants were stored in aliquots at −80 ◦C until assays. Platelet poor plasma for the assessment of thrombin generation was obtained by double centrifugation at 2000 ×g for 15 min

at room temperature and stored at − 80 °C until analysis. Thrombin generation was evaluated by using the Calibrated Automated Thrombogram (CAT) (Thrombinoscope BV).

#### Calibrated Automated Thrombogram (CAT) assessment

We used the method described by Hemker and coworkers17 and commercialized by Thrombinoscope BV, (Maastricht, the Netherlands). According to the manufacturer's instructions, measurements were conducted in 80 µl of platelet poor plasma (PPP) mixed with 20 µl PPP-reagent, containing 5 pmol/L tissue factor and 4 µmol/L phospholipids in 96-well microtiter plates. PPP samples were run in duplicate for the measurement of TG. In order to correct for inner filter effects and substrate consumption, each thrombin generation measurement was calibrated against the fluorescence curve obtained in the same plasma to which a fixed amount of thrombin-α2-macroglobulin complex was added (20 µl Thrombin Calibrator; Thrombinoscope BV). The reaction was started adding 20 µl of fluorogenic substrate to sample and calibrator wells and then the fluorescence intensity was detected in a Fluoroskan Ascent reader (Thermo Labsystems OY, Helsinki, Finland) equipped with a 390/460 filter set for 60 minutes and the thrombin generation curves were calculated with Thrombinoscope software (Thrombinoscope BV). TG was expressed as endogenous thrombin potential (ETP: the area under the curve that represents the total amount of thrombin generated), peak (the maximum concentration of thrombin produced), and velocity index (the slope between the start of thrombin formation and the peak).

## 3.4 Fibrinolysis markers

### D-Dimer

D-Dimer (DD) plasma concentrations were assessed by using a commercial kit (VIDAS® D-Dimer) which is a highly sensitive automated test for the immunoenzymatic determination of fibrin degradation products in human plasma.

### Clot Lysis Time

The lysis of a tissue factor induced clot by exogenous t-PA (CLT) was studied by monitoring changes in turbidity during clot formation and subsequent lysis according to Lisman.[91]

## 3.5 Von Willebrand factor/ADAMTS-13

### ADAMTS-13 Activity assay

The HemosIL AcuStar ADAMTS-13 Activity assay is a two-step immunoassay to quantify ADAMTS-13 Activity in human citrated plasma using magnetic particles as solid phase and a chemiluminescent detection system. In the first step, the sample is mixed with the assay buffer and magnetic particles coated with a recombinant GST-VWF73 peptide substrate containing the ADAMATS-13 tyr1605- Met1606 cleavage site by means of a specific monoclonal anti-Glutathione S-Transferase (GST) antibody. The ADAMTS-13 present in the sample cleaves the substrate bound to magnetic particles proportionally to its activity. After

magnetic separation and washing, a monoclonaly antibody labeled with isoluminol that reacts specifically with the cleaved peptide (anti-N10) is added and incubated in a second step. After this second incubation, a magnetic separation and a wash step, reagents that trigger the chemiluminescent reaction are added. The emitted light is proportional to the ADAMTS-13 activity in the sample and is measured as relative light units (RLU) by the AcuStar luminometer.

### Von Willebrand Factor assay

The VWF Activity kit (Werfen, Italy) is a latex particle enhanced immunoturbidimetric assay to quantify VWF Activity in plasma. The activity of VWF is determined by measuring the increase of turbidity produced by the agglutination of the latex reagent. A specific anti-VWF monoclonal antibody adsorbed onto the latex reagent, directed against the platelet binding site of VWF (Glycoprotein Ib receptor), reacts with the VWF of patient plasma. The degree of agglutination is directly proportional to the activity of VWF in the sample and is determined by measuring the decrease of transmitted light caused by the aggregates.

## 3.6 Markers of oxidative stress

### TBARS (Thiobarbituric Acid Reactive Substances) estimation

Plasma TBARS levels were measured using a TBARS assay kit in accordance with the manufacturer's instructions. Briefly, the adduct generated by reacting malondialdehyde with Thiobarbituric acid after 1h at 95 ºC was measured spectrofluorimetrically, with excitation at 530 nm and emission at 550 nm. TBARS was expressed in terms of malondialdehyde equivalent (nmol/ml) and then normalized for protein concentration.

### Total antioxidant capacity (TAC) assay

The ORAC method (Oxygen Radical Absorbance Capacity), based on the inhibition of the peroxylradical-induced oxidation initiated by thermal decomposition of azo-compounds, like 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH), was performed as previously described.[92] Briefly, a fluorescein solution prepared daily was used. Trolox was used as a standard. Sample with fluorescein was pre-incubated for 30 min at 37 °C in each well, before rapidly adding AAPH solution. Fluorescence was measured with excitation at 485 nm and emission at 537 nm in a Fluoroskan Ascent Microplate Fluorometer. Results were expressed as Trolox Equivalents (μM) and then normalized for protein concentration.

## 3.7 DOAC plasmatic concentrations

### Dabigatran assay

Dabigatran circulating plasma levels were assessed by a commercial kit - HemosIL DTI assay. This assay is a modified (dilute) thrombin time test performed on citrated patient plasma. Citrated patient plasma is diluted in pooled normal plasma (DTI Plasma Diluent – supplied as part of the assay) to reduce interferences

from pre-analytical variables. A fixed concentration of reconstituted bovine thrombin is then added to the diluted patient sample, activating the coagulation cascade and converting fibrinogen into fibrin. The presence of Dabigatran in patient samples will have an inhibitory effect on the procoagulant activity of the exogenous thrombin added to the patient sample. The associated clotting time in seconds is measured on the ACL TOP Family. A Dabigatran reference curve is plotted on the ACL TOP Family from the clotting time results of a known reference plasma standard (HemosIL Dabigatran Calibrators). The concentration of Dabigatran in the patient plasma samples is determined by comparing clotting time values to the reference curve.

### Apixaban, Edoxaban and Rivaroxaban assay

Rivaroxaban, Edoxaban and Apixaban plasma concentrations were assassed by a commercial kit -Hemosil Liquid Anti-Xa. This kit is a one stage chromogenic assay based on a synthetic chromogenic substrate and on Factor Xa inactivation. Rivaroxaban, Edoxaban and Apixaban levels in patient plasma are measured automatically on ACL TOP Family Systems when this assay is calibrated with the HemosIL Rivaroxaban, Edoxaban and Apixaban Calibrators respectively. Rivaroxaban, Edoxaban and Apixaban directly inhibit Factor Xa activity independent of the antithrombin present. Residual Factor Xa is quantified with a synthetic chromogenic substrate S-2732. The paranitroaniline released is monitored kinetically at 405 nm and is inversely proportional to the Rivaroxaban, Edoxaban and Apixaban levels in the sample.

## 4. Follow-up and endpoints

Follow-up was performed at 1, 3, 6 and 12 months mainly by telephone or clinical evaluation in some cases.

The study aims to evaluate in a real-life population: 1) different clinical choices in AFpatients who underwent PCI; 3) a possible association between laboratory markers and clinical events.

The endpoints were the following.

1) The primary endpoint of the study was to evaluate the incidence of major and minor haemorrhagic events, according to ISTH classification (International Society of Thrombosis and Haemostasis),[93] table 2.

2) The secondary endpoint was the incidence of major cardiovascular and cerebrovascular events (MACCE: IMA, stent thrombosis, percutaneous revascularization, ischemic stroke, cardiovascular death)].

Myocardial infarction was defined according to the fourth universal definition of 2018 ESC guidelines.[55] Ischemic stroke was defined as an episode of neurological dysfunction caused by focal cerebral, spinal, or retinal infarction persisting ≥24 hours or until death.[94]

Table 2. ISTH classification

| | |
|---|---|
| Major (non-surgical patients) | 1. Fatal bleeding.<br>and/or<br>2. Symptomatic bleeding in a critical area or organ, such as intracranial, intraspinal, intraocular, retroperitoneal, intraarticular, or pericardial, or intramuscular with compartment syndrome.<br>and/or<br>3. Bleeding causing a fall in hemoglobin level of 2 g/dL (1.24 mmol/L) or more, or leading to transfusion of two or more units of whole blood or red cells. |
| Minor clinically relevant | A clinically relevant minor bleed is an acute or subacute clinically overt bleed that does not meet the criteria for a major bleed but prompts a clinical response, in that it leads to at least one of the following:<br>• A hospital admission for bleeding, or<br>• A physician guided medical or surgical treatment for bleeding, or<br>• A change in antithrombotic therapy (including interruption or discontinuation of study drug). |
| Minor | All non-major bleeds that do not meet the criteria for a clinically relevant bleeding. |

Moreover, at each step of follow-up, antithrombotic therapy modifications were recorded. Follow-up was interrupted after the occurrence of an adverse event, death, or because of explicit denial of patients to give information.

# 5. Statistical analysis

Statistical analysis was performed with SPSS (Statistical Package for Social Sciences, Chicago, Illinois, USA), version 25.0. Categorical data were reported as frequencies. Continuous variables were reported as mean and standard deviation or median and interquartile range, as appropriate (Kolmogorov Smirnov test was performed). Dichotomic variables were compared through Chi square test or Fisher exact Test, while continuous variable by T student or Mann-Whitney. Cumulative survival curves were made with Kaplan-Meier method. To explore the presence of association between variables and events Cox regression analysis was used.

# RESULTS

## 1. Baseline characteristics

A total of 147 patients were included in the study; the mean age was 78±8 years and 48 (33%) were women. The most common CAD manifestation was ACS (n=103, 70%) and in about 25% of patients, AF was diagnosed for the first time during index hospitalization. Four patients had a mechanical prosthetic valve, too. The antithrombotic therapy at discharge was DAT in 56 cases (38%) e TAT in 91 (62%) (Figure 1).

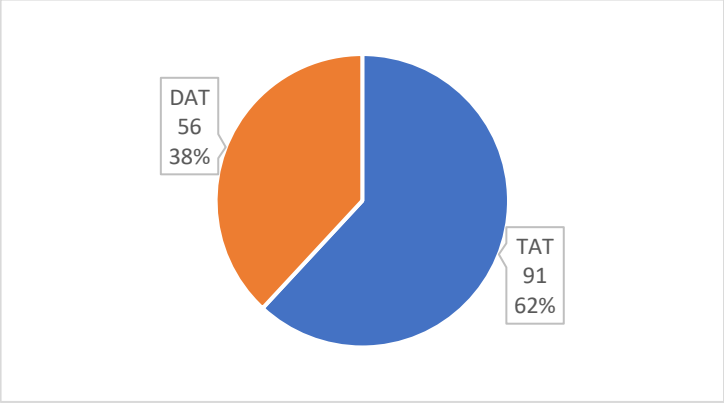



**Figure 1**. Antithrombotic therapy in the study population (DAT=Dual Antithrombotic Therapy; TAT=Triple Antithrombotic Therapy)

Among patients in TAT, the anticoagulant of choice was DOAC in 53 cases (58%); dabigatran and apixaban were the most adopted (figure 2.1). All patients but 2 received clopidogrel as $P2Y_{12}$ inhibitor of choice, while in the other cases ticagrelor was prescribed.

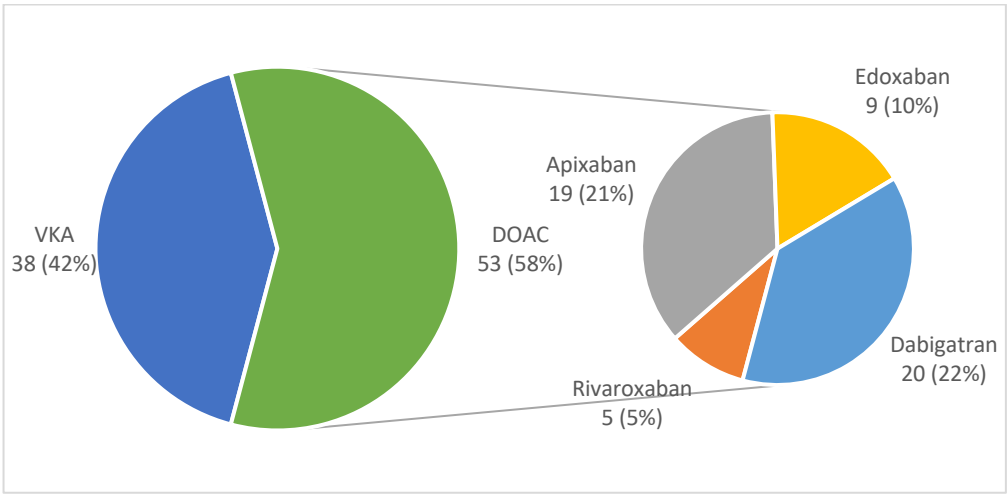



**Figure 2.1** Type of anticoagulant prescribed in patients discharged in TAT (DOAC: direct oral anticoagulant; VKA: vitamin K antagonist).

Among patients on DAT, DOAC were even more prescribed (47 cases, 84%) (figure 2.2). In this group the antiplatelet drug of choice was clopidogrel in most cases: ticagrelor was prescribed in 6 patients (10.7%) and aspirin in one (0.7%).

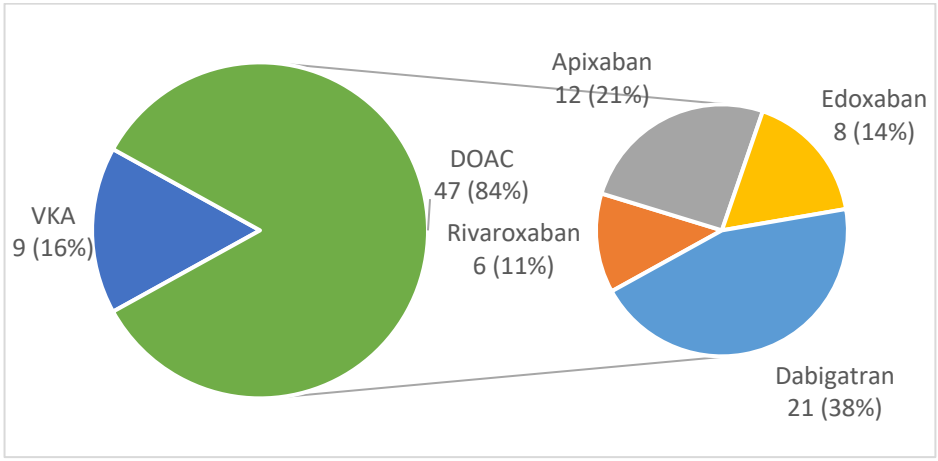



**Figure 2.2.** Type of anticoagulant prescribed in patients discharged in DAT. DOAC: direct oral anticoagulant; VKA: vitamin K antagonist.

Table 1.1 shows baseline characteristics of the study population according to antithrombotic pattern. Patients on TAT had a significantly lower BMI, a more complex CAD in terms of number of diseased and treated vessels and a worse renal function; in particular, all patients in dialysis were discharged in TAT.

**Table 1.1** Baseline characteristics of the study population according to antithrombotic pattern.

| | Total (n=147) | TAT (n=91) | DAT (n=56) | P value |
|---|---|---|---|---|
| **Age** [yrs], (mean±SD) | 78±8 | 77±8 | 79±8 | 0.093 |
| **Male sex,** n (%) | 99 (67.3) | 62 (68.1) | 37 (66.1) | 0.796 |
| **Hypertension,** n (%) | 122 (83.0) | 74 (81.3) | 48 (85.7) | 0.491 |
| **Dyslipidemia**, n (%) | 86 (58.5) | 58 (63.7) | 28 (50.0) | 0.101 |
| **Smokers**, n (%)<br>Former Smokers, n (%) | 16 (10.9)<br>58 (39.4) | 11 (12.1)<br>39 (42.9) | 5 (8.9)<br>19 (33.9) | 0.361 |
| **Diabetes mellitus**, n (%) | 50 (34.0) | 34 (37.4) | 16 (28.6) | 0.275 |
| **Family history of CVD**, n (%) | 22 (15.0) | 15 (16.5) | 7 (12.5) | 0.511 |
| **BMI** (mean±SD) | 27.0±4.0 | 26.3±3.5 | 28.0±4.5 | 0.014 |
| **Prior MI**, n (%) | 53 (36.0) | 33 (36.3) | 20 (35.7) | 0.946 |

| | | | | |
|---|---|---|---|---|
| **Prior PCI**, n (%) | 64 (43.5) | 41 (45.1) | 23 (41.1) | 0.636 |
| **Prior CABG**, n (%) | 12 (8.2) | 8 (8.8) | 4 (7.1) | 0.723 |
| **Prior TIA/stroke,** n (%) | 18 (12.2) | 11 (12.1) | 7 (12.5) | 0.941 |
| **PAD,** n (%) | 48 (32.7) | 28 (30.8) | 20 (35.7) | 0.589 |
| **Prior bleeding,** n (%) | 12 (8.2) | 5 (5.5) | 7 (12.5) | 0.212 |
| **ACS,** n (%) | 103 (70.1) | 68 (74.7) | 35 (62.5) | 0.116 |
| **UA,** n (%)<br>**NSTEMI,** n (%)<br>**STEMI,** n (%) | 27 (18.4)<br>55 (37.4)<br>21 (14.3) | 22 (24.2)<br>33 (36.3)<br>13 (14.3) | 5 (8.9)<br>22 (39.3)<br>8 (14.3) | 0.141 |
| **MI** | 66 (44.9) | 46 (50.1) | 20 (53.5) | 0.048 (vs UA) |
| **LVEF** [%], (mean ± SD) | 46±11 | 45±11 | 48±11 | 0.087 |
| **Number of diseased vessels** **1** n (%)<br>**2** n (%)<br>**3** n (%) | 2 (1-3)<br>45 (30.6)<br>55 (37.4)<br>46 (31.3) | 2 (1-3)<br>17 (18.9)<br>38 (42.2)<br>35 (38.9) | 1.5 (1-2)<br>28 (50.0)<br>17 (30.4)<br>11 (19.6) | <0.001 |
| **LM disease, n (%)** | 41 (27.9) | 32 (35.6) | 9 (16.1) | 0.011 |
| **Number of treated vessels,** median (IQR) | 1 (1-2) | 1 (1-2) | 1 (1-2) | 0.020 |
| **Number of stent,** median (IQR) | 2 (1-3) | 2 (1-3) | 1 (1-2) | 0.016 |
| **Total stent length,** mm (mean±SD) | 49.2±34.1 | 53.4±3.7 | 41.4±32.1 | 0.054 |
| **$CHA_2DS_2$-VASc score** (mean±SD) | 4.7±1.2 | 4.8±1.4 | 4.7±11.2 | 0.924 |
| **HAS-BLED score** (mean±SD) | 2.4±0.7 | 2.5±0.7 | 2.3±0.6 | 0.695 |
| **Statin,** n (%)<br>**Atorvastatin,** n (%)<br>**Rosuvastatin,** n (%) | 128 (87.0)<br>54 (69.4)<br>16 (10.9) | 82 (90.1)<br>70 (76.9)<br>9 (9.9) | 46 (82.1)<br>32 (57.1)<br>7 (12.5) | 0.207 |

| | | | | |
|---|---|---|---|---|
| **Pravastatin,** n (%)<br>**Simvastatin,** n (%) | 1 (0.7)<br>9 (6.1) | 0 (0)<br>3 (3.3) | 1 (1.8)<br>6 (10.7) | |
| **PPI,** n (%)<br>**Lansoprazol,** n (%)<br>**Omeprazol,** n (%)<br>**Pantoprazol,** n (%) | 136 (92.5)<br>25 (17.0)<br>3 (2.0)<br>108 (73.5) | 85 (93.4)<br>14 (15.4)<br>2 (2.2)<br>69 (75.8) | 51 (91.1)<br>11 (19.6)<br>1 (1.8)<br>39 (69.6) | 0.749 |
| **Hb** [g/dL], (mean±SD) | 12.0±2.5 | 11.8±1.9 | 12.3±3.3 | 0.224 |
| **Platelets** [/mL], (mean±SD) | 221360±85048 | 220813±87247 | 222250±82119 | 0.921 |
| **MCV** (mean±SD) | 89.3±8.1 | 89.1±9.0 | 89.8±6.3 | 0.597 |
| **Creatinin** [mg/dL], (mean±SD) | 1.4±0.9 | 1.6±1.2 | 1.1±0.3 | 0.001 |
| **eGFR** [ml/min], (mean±SD) | 52±23 | 48±24 | 57±22 | 0.023 |
| **Dialysis,** n (%) | 7 (4.7) | 7 (10.6) | 0 | 0.035 |

Abbreviations: BMI, body mass index; CABG, coronary artery bypass grafting; CVC, cardiovascular disease; SD, standard deviation; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; Hb, Hemoglobin; MCV, mean cellular volume; MI; myocardial infarction; UA, unstable angina; STEMI, ST elevation myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; IQR, interquartile range; PCI, percutaneous coronary intervention; PPI, proton pump inhibitor; LM, left main.

As concerns the anticoagulant therapy, a VKA in TAT was preferred in younger patients and in those with a previous stroke or TIA; patients in this group had also a higher mean HASBLED score and a lower mean eGFR as compared to those in DOAC-TAT group (Table 1.2). No statistic difference was observed for other variables among patients in DAT.

**Table 1.2** Baseline characteristics of the study population according to antithrombotic pattern and type of anticoagulant.

| | **TAT** | | | **DAT** | | |
|---|---|---|---|---|---|---|
| | **VKA (n=38)** | **DOAC (n=53)** | **P value** | **VKA (n=9)** | **DOAC (n=47)** | **P value** |
| **Age** [yrs], (mean±SD) | 74±9 | 78±6 | 0.011 | 80±8 | 79±7 | 0.521 |
| **Male sex,** n (%) | 28 (73.7) | 34 (64.2) | 0.336 | 6 (66.7) | 31 (66) | 0.967 |
| **Hypertension,** n (%) | 32 (84.2) | 42 (79.2) | 0.542 | 9 (100) | 39 (83) | 0.181 |
| **Dyslipidemia**, n (%) | 27 (71.1) | 31 (58.5) | 0.219 | 5 (55.6) | 23 (48.9) | 0.716 |
| **Smokers**, n (%) | 5 (13.2) | 6 (11.3) | 0.663 | 1 (11.1) | 4 (8.5) | 0.969 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Former Smokers, n (%) | 18 (47.4) | 21 (39.6) | | 3 (33.3) | 16 (34) | |
| **Diabetes mellitus**, n (%) | 18 (47.4) | 16 (30.2) | 0.095 | 2 (22.2) | 14 (29.8) | 0.645 |
| **Family history of CVD**, n (%) | 6 (15.8) | 9 (17) | 0.880 | 0 | 7 (14.9) | 0.216 |
| **BMI** (mean±SD) | 25.7±3.6 | 26.7±3.5 | 0.240 | 27.3±3.5 | 28.1±4.7 | 0.633 |
| **Prior MI**, n (%) | 12 (31.6) | 21 (39.6) | 0.431 | 2 (22.2) | 18 (38.3) | 0.356 |
| **Prior PCI**, n (%) | 16 (42.1) | 25 (47.2) | 0.632 | 2 (22.2) | 21 (44.7) | 0.210 |
| **Prior CABG**, n (%) | 5 (13.2) | 3 (5.7) | 0.213 | 1 (11.1) | 3 (6.4) | 0.614 |
| **Prior TIA/stroke,** n (%) | 9 (23.7) | 2 (3.8) | 0.004 | 2 (22.2) | 5 (10.6) | 0.336 |
| **PAD,** n (%) | 14 (36.8) | 14 (26.4) | 0.413 | 3 (33.3) | 17 (36.2) | 0.723 |
| **Prior bleeding,** n (%) | 0 | 5 (9.4) | 0.051 | 1 (11.1) | 6 (12.8) | 0.891 |
| **ACS,** n (%) | 31 (81.6) | 37 (69.8) | 0.203 | 5 (55.6) | 30 (63.8) | 0.639 |
| **UA,** n (%) | 8 (21.1) | 14 (26.4) | 0.043 | 1 (11.1) | 4 (8.5) | 0.166 |
| **NSTEMI,** n (%) | 13 (34.2) | 20 (37.7) | | 1 (11.1) | 21 (44.7) | |
| **STEMI,** n (%) | 10 (26.3) | 3 (5.7) | | 3 (33.3) | 5 (10.6) | |
| **LVEF** [%], (mean ± SD) | 45±11 | 45±11 | 0.770 | 44±12 | 49±11 | 0.193 |
| **N of diseased vessels**<br>**1** n (%)<br>**2** n (%)<br>**3** n (%) | 2 (2-3)<br>8 (21.1)<br>16 (42.1)<br>14 (36.8) | 2 (2-3)<br>9 (17)<br>22 (41.5)<br>21 (39.6) | 0.653 | 1 (1-2)<br>6 (66.7)<br>2 (22.2)<br>1 (11.1) | 1 (1-2)<br>22 (46.8)<br>15 (31.9)<br>10 (21.3) | 0.279 |
| **LM disease, n (%)** | 16 (43.2) | 16 (30.2) | 0.203 | 3 (33.3) | 6 (12.8) | 0.124 |
| **N of treated vessels,** median (IQR) | 2 (1-2) | 1 (1-2) | 0.563 | 1 (1-1) | 1 (1-2) | 0.211 |
| **N stent** median (IQR) | 2 (1-3) | 2 (1-3) | 0.944 | 1 (1-2) | 1 (1-2) | 0.933 |
| **Total stent length,** mm (mean±SD) | 52±30 | 54±37 | 0.810 | 47±29 | 40±33 | 0.599 |
| **$CHA_2DS_2$-VASc score** (mean±SD) | 5.0±1.6 | 4.7±1.2 | 0.263 | 5.1±1.1 | 4.6±1.2 | 0.277 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **HAS-BLED score** (mean±SD) | 2.8±0.8 | 2.1±0.5 | 0.010 | 2.4±0.5 | 2.3±0.6 | 0.469 |
| **Statin,** n (%)<br>**Atorvastatin,** n (%)<br>**Rosuvastatin,** n (%)<br>**Pravastatin,** n (%)<br>**Simvastatin,** n (%) | 33 (86.8)<br>33 (86.8)<br>0<br>0<br>0 | 49 (92.4)<br>37 (69.8)<br>9 (17)<br>0<br>3 (5.7) | 0.483 | 6 (66.7)<br>5 (55.6)<br>0<br>0<br>1 (11.1) | 40 (85.1)<br>27 (57.4)<br>1 (2.1)<br>7 (14.9)<br>5 (10.6) | 0.338 |
| **PPI,** n (%)<br>**Lansoprazol,** n (%)<br>**Omeprazol,** n (%)<br>**Pantoprazol,** n (%) | 34 (89.5)<br>7 (18.4)<br>2 (5.3)<br>25 (65.8) | 51 (96.2)<br>7 (13.2)<br>0<br>44 (83) | 0.231<br><br><br>0.134 | 8 (88.9)<br>1 (11.1)<br>0<br>7 (77.8) | 43 (91.5)<br>10 (21.3)<br>1 (2.1)<br>32 (68.1) | 0.999<br><br><br>0.862 |
| **Hb** [g/dL], (mean±SD) | 11.4±2.0 | 12.1±1.8 | 0.097 | 13.7±7.0 | 12.0±2.0 | 0.490 |
| **Platelets** [/mL], (mean±SD) | 222447± 71673 | 219641± 97551 | 0.881 | 220222± 108587 | 222638± 77530 | 0.936 |
| **MCV** (mean±SD) | 89.3±12.1 | 89.0±6.1 | 0.877 | 90.6±3.0 | 89.7±6.8 | 0.701 |
| **Creatinin** [mg/dL], (mean±SD) | 2.2±1.6 | 1.1±0.4 | <0.001 | 1.1±0.3 | 1.1±0.3 | 0.585 |
| **eGFR** [ml/min], (mean±SD) | 41±27 | 53±20 | 0.017 | 51±19 | 58±23 | 0.349 |
| **Dialysis,** n (%) | 7 (18.4) | 0 | 0.001 | 0 | 0 | - |

Abbreviations: BMI, body mass index; CABG, coronary artery bypass grafting; CVC, cardiovascular disease; SD, standard deviation; eGFR, estimated glomerular filtration rate; LVEF, left ventricular ejection fraction; Hb, Hemoglobin; MCV, mean cellular volume; MI; myocardial infarction; UA, unstable angina; STEMI, ST elevation myocardial infarction; NSTEMI, non-ST elevation myocardial infarction; IQR, interquartile range; PCI, percutaneous coronary intervention; PPI, proton pump inhibitor; LM, left main.

# 2. Laboratory analyses

Table 2.1 displays laboratory parameters results. The only significant difference between TAT and DAT group was observed in mean levels of thromboxane A2 (TXA2)- dependent platelet reactivity, as expected.

**Table 2.1** Laboratory test results according to the type of antithrombotic pattern.

| | **Total** (n=147) | **TAT** (n=91) | **DAT** (n=56) | **P value** |
|---|---|---|---|---|
| **Platelet aggregation parameters** | | | | |

| | | | | |
|---|---|---|---|---|
| **ADP test in citrate [U],** median (IQR) | 26 (20-35) | 26 (20-32) | 26 (17-38) | 0.937 |
| **ASPI test in citrate [U],** median (IQR) | 18 (12-26) | 17 (12-22) | 18 (14-37) | 0.086 |
| **TXA2 dependent platelet reactivity [ARU]** (mean±SD) | 485±98 | 457±95 | 533±83 | <0.001 |
| **$P2Y_{12}$ dependent platelet reactivity [PRU]** (mean±SD) | 173±88 | 176±88 | 167±89 | 0.557 |
| **ADAMTS-13** (mean±SD) | 72±24 | 75±22 | 66±25 | 0.069 |
| **VWF,** median (IQR) | 224 (207-233) | 223 (206-233) | 225 (207-232) | 0.590 |
| **VWF/ADAMTS-13,** median (IQR) | 3.1 (2.3-4.2) | 3.1 (2.2-4.0) | 3.4 (2.5-4.4) | 0.153 |
| **Redox balance**<br>**ORAC** (mean±SD)<br>**TBARS** (mean±SD) | <br>17.3±8.8<br>0.7±0.4 | <br>17.9±9.0<br>0.7±0.4 | <br>16.5±8.6<br>0.6±0.4 | <br>0.372<br>0.259 |
| **Coagulation parameters** | | | | |
| **ETP without thrombomodulin [nM x min],** median (IQR) | 619 (362-1124) | 973 (632-1676) | 1106 (690-1549) | 0.751 |
| **ETP with thrombomodulin [nM x min],** median (IQR) | 1048 (637-1637) | 632 (362-1247) | 607 (362-999) | 0.893 |
| **Thrombin generation peak [nM],** median (IQR) | 200 (118-284) | 132 (60-246) | 130 (62-236) | 0.923 |
| **TG time-to peak [min],** median (IQR) | 11.5 (8.3-14.7) | 11.7 (8.1-14.6) | 11.5 (8.5-15.7) | 0.907 |
| **TG velocity index [nm/min],** median (IQR) | 40.1 (14.7-98.4) | 41.1 (14.6-106.1) | 37.1 (15.0-82.4) | 0.968 |
| **TG lag time [min],** median (IQR) | 7.5 (5.3-9.6) | 7.5 (5.3-9.6) | 7.6 (5.7-9.7) | 0.923 |
| **ETP ratio,** median (IQR) | 0.66 (0.42-0.887 | 0.67 (0.42-0.88) | 0.66 (0.42-0.85) | 0.987 |
| **INR,** median (IQR) | 1.3 (1.2-1.6) | 1.3 (1.2-1.7) | 1.3 (1.2-1.4) | 0.471 |
| **aPTT [sec]** (mean±SD) | 35±11 | 36±13 | 33±7 | 0.062 |

| | | | | |
|---|---|---|---|---|
| **Fibrinolysis parameters** | | | | |
| **D-Dimer [ng/mL FEU],** median (IQR) | 963 (501-1670) | 995 (493-1701) | 870 (508-1632) | 0.848 |
| **Clot lysis time [min**], median (IQR) | 72 (61-83) | 73 (62-83) | 70 (59-84) | 0.477 |
| **Hemorheology parameters** | | | | |
| **Elongation index** (mean±SD) | 0.393±0.027 | 0.395±0.026 | 0.390±0.028 | 0.437 |
| **Erythrocyte aggregation index [%],** median (IQR) | 69 (63-73) | 69 (64-72) | 69 (62-75) | 0.962 |
| **DOAC plasmatic concentration** | | | | |
| **Dabigatran** [ng/mL] (mean±SD) | N=29<br>74±44 | N=20<br>8±47 | N=9<br>69±49 | 0.487 |
| **Apixaban** [ng/mL] (mean±SD) | N=24<br>143±69 | N=19<br>127±73 | N=5<br>163±59 | 0.138 |
| **Rivaroxaban** [ng/mL] (mean±SD) | N=7<br>86±81 | N=5<br>100±112 | N=2<br>75±53 | 0.663 |
| **Edoxaban** [ng/mL] (mean±SD) | N=15<br>58±41 | N=9<br>65±48 | N=6<br>53±33 | 0.622 |

Abbreviations: DOAC, direct oral anticoagulant; ETP, endogenous thrombin potential; SD, standard deviation; IQR, interquartile range; TG, thrombin generation; INR, international normalized ratio; aPTT activated partial thromboplastin time, VWF, Von Willebrand factor.

Apart from expected difference in mean INR between patients discharged with DOAC and VKA, a higher ETP was observed in DOAC patients both in TAT and in DAT (table 2.2). The TAT-DOAC group was characterized by a lower level of oxidative stress, as expressed by a higher mean ORAC value (19.6±10.0 vs 15.8±6.9; p= 0.048).

**Table 2.2** Laboratory test results according to the type of antithrombotic pattern and anticoagulant drug.

| | TAT | | | DAT | | |
|---|---|---|---|---|---|---|
| | VKA (n=38) | DOAC (n=53) | P value | VKA (n=9) | DOAC (n=47) | P value |
| **Platelet aggregation parameters** | | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **ADP test in citrate [U],** median (IQR) | 28 (21-36) | 23 (20-29) | 0.116 | 27 (16-41) | 25 (17-36) | 0.793 |
| **ASPI test in citrate [U],** median (IQR) | 18 (12-25) | 17 (11-21) | 0.629 | 22 (11-47) | 18 (14-35) | 0.909 |
| **TXA2 dependent platelet reactivity [ARU]** (mean±SD) | 459±99 | 455±93 | 0.859 | 514±88 | 537±83 | 0.441 |
| **$P2Y_{12}$ dependent platelet reactivity [PRU]** (mean±SD) | 185±83 | 169±91 | 0.372 | 162±93 | 168±89 | 0.846 |
| **ADAMTS-13** (mean±SD) | 71±26 | 77±20 | 0.338 | 66±18 | 66±27 | 0.978 |
| **VWF,** median (IQR) | 221 (209-230) | 224 (198-233) | 0.975 | 231 (215-236) | 224 (206-230) | 0.179 |
| **VWF/ADAMTS-13,** median (IQR) | 3.2 (2-3-4.1) | 2.8 (2.1-3.7) | 0.181 | 3.9 (2.5-4.7) | 3.4 (2.5-4.2) | 0.725 |
| **Redox balance**<br>**ORAC** (mean±SD)<br>**ORAC ratio** (mean±SD)<br>**TBARS** (mean±SD) | <br>15.8±6.9<br>0.7±0.4<br>0.9±0.2 | <br>19.6±10.0<br>0.6±0.4<br>1.1±0.5 | <br>0.048<br>0.254<br>0.037 | <br>15±12<br>0.7±0.3<br>1.2±1.1 | <br>17±8<br>0.6±0.4<br>0.9-0.3 | <br>0.701<br>0.515<br>0.494 |
| **Coagulation parameters** | | | | | | |
| **ETP without thrombomodulin [nM x min],** median (IQR) | 639 (286-944) | 1400 (909-2260) | <0.001 | 478 (97-774) | 1182 (874-1647) | 0.008 |
| **ETP with thrombomodulin [nM x min],** median (IQR) | 556 (165-795) | 837 (405-1901) | 0.014 | 441 (81-873) | 696 (404-1107) | 0.223 |
| **TG peak [nM],** median (IQR) | 116 (41-197) | 172 (62-456) | 0.012 | 91 (13-148) | 147 (63-296) | 0.186 |
| **TG time-to peak [min],** median (IQR) | 11.0 (7.7-15.0) | 12.2 (8.5-14.5) | 0.728 | 12.8 (8.2-78.4) | 11.3 (8.5-15.7) | 0.422 |
| **TG velocity index [nm/min],** median (IQR) | 36 (8.8-65.4) | 42.2 (17.8-176.5) | 0.105 | 36.7 (9.5-52.9) | 40.1 (15.5-98.1) | 0.760 |
| **TG lag time [min],** median (IQR) | 7.3 (4.8-10.8) | 7.8 (5.3-9.4) | 0.640 | 9.0 (5.7-77.7) | 7.4 (5.7-9.4) | 0.183 |
| **ETP ratio,** median (IQR) | 0.85 (0.50-0.91 | 0.59 (0.41-0.85) | 0.139 | 0.93 (0.28-1.06) | 0.63 (0.42-0.80) | 0.126 |

| | | | | | | |
|---|---|---|---|---|---|---|
| **INR,** median (IQR) | 1.7 (1.3-1.8) | 1.3 (1.2-1.5) | <0.001 | 1.8 (1.3-2.8) | 1.3 (1.2-1.4) | 0.002 |
| **aPTT [sec]** (mean±SD) | 37±16 | 34±9 | 0.316 | 34±7 | 32±7 | 0.456 |
| **Fibrinolysis parameters** | | | | | | |
| **D-Dimer [ng/mL FEU],** median (IQR) | 1247 (445-1713) | 916 (529-1578) | 0.515 | 1235 (487-2462) | 866 (513-1266) | 0.532 |
| **Clot lysis time [min],** median (IQR) | 71 (63-80) | 74 (61-83) | 0.529 | 75 (56-89) | 69 (59-84) | 0.691 |
| **Hemorheology parameters** | | | | | | |
| **Elongation index** (mean±SD) | 0.390±0.022 | 0.400±0.030 | 0.121 | 0.394±0.023 | 0.390±0.028 | 0.780 |
| **Erythrocyte aggregation index [%],** median (IQR) | 69 (66-74) | 68 (63-72) | 0.282 | 63 (60-68) | 70 (62-75) | 0.231 |

Abbreviations: DOAC, direct oral anticoagulant; SD, standard deviation; IQR, interquartile range; VKA, vitamin K antagonist; ETP, endogenous thrombin potential; TG, thrombin generation; INR, international normalized ratio; aPTT activated partial thromboplastin time, VWF, Von Willebrand factor.

# 3. Follow-up

Follow-up was performed at predefined timepoints (1, 3, 6 e 12 months since enrollment); in the present analysis were considered those patients who completed at least 1-month follow-up (median 203 days, IQR 115-378). As concerns the antithrombotic therapy, in TAT subgroup aspirin was interrupted after one week in 17 patients, after 1 month in 26 and after 3 months in 25. Among the 86 subjects that reached a 6-months follow-up, 21 (24%) still received TAT (figure 3.1).

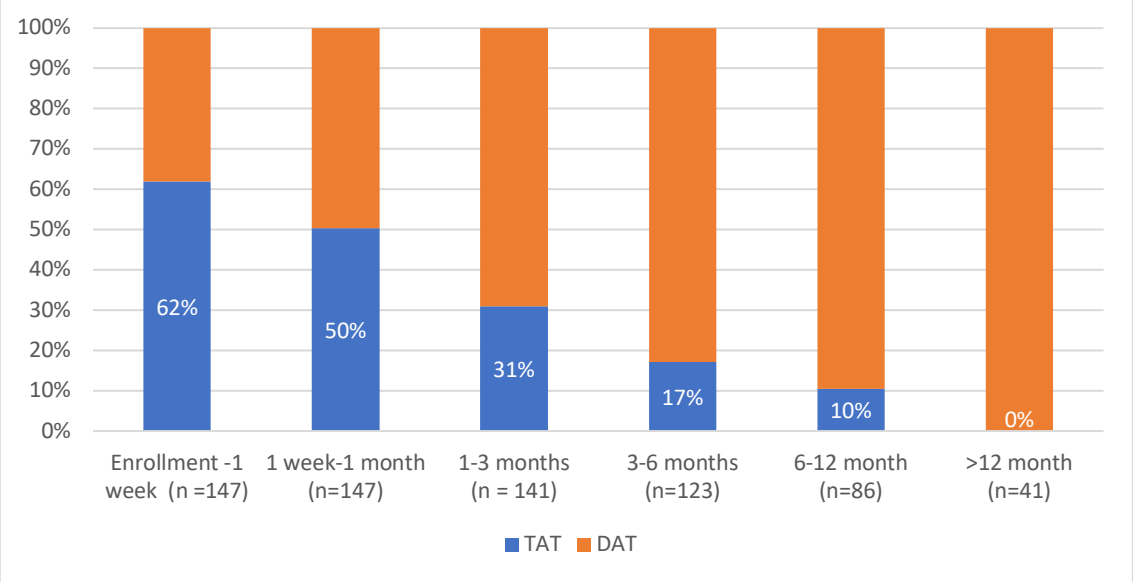



**Figure 3.1.** Antithrombotic pattern during follow-up.

The incidence of the adverse events that occurred during follow-up are shown in table 3. Major adverse cardiac and cerebrovascular events (MACCE) occurred in 25 cases (17%), while hemorrhagic events in 23 cases (15.6%), 13 of which were major (8.8%). The incidence of all-cause death was 12.2% (18 patients); 11 subjects died from cardiovascular causes (7.4%).

**Table 3.** Adverse events according to antithrombotic therapy pattern.

| | TAT | | DAT | |
|---|---|---|---|---|
| | VKA (n=38) | DOAC (n=53) | VKA (n=9) | DOAC (n=47) |
| **MACCE** | | | | |
| ACS, n (%) | 4 (10.5) | 7 (13.2) | | 3 (6.4) |
| Critical limb ischemia, n (%) | 1 (2.6) | | | |
| Mesenteric ischemia, n (%) | | | | 1 (2.1) |
| Ischemic stroke, n (%) | 2 (5.3) | 1 (1.9) | | |
| Fatal MACCE, n (%) | 1 (2.6) | 2 (3.8) | | 3 (6.4) |
| **Bleeding events** | | | | |
| **Major** | | | | |
| ICH, n (%) | | 2 (3.8) | | 1 (2.1) |
| Fatal ICH, n (%) | | 1 (1.9) | | |
| GI bleeding, n (%) | | 3 (5.7) | 2 (22.2) | 2 (4.3) |
| GU bleeding, n (%) | 1 (2.6) | | | |
| Procedural, n (%) | | 1 (1.9) | | |
| **Non major clinically relevant,** n (%) | 1 (2.6) | 5 (9.4) | | |
| **Minor,** n (%) | 1 (2.6) | 1 (1.9) | | 2 (4.3) |
| **Death from any cause,** n (%) | 6 (15.8) | 4 (7.5) | 3 (33.3) | 5 (10.6) |
| **Death from cardiovascular cause,** n (%) | 4 (10.5) | 3 (5.7) | 1 (11.1) | 3 (6.4) |

Abbreviations: DOAC, oral direct anticoagulant; GI, gastrointestinal; GU, genitourinary; ICH, intracerebral hemorrhage; ACS, acute coronary syndrome, VKA, vitamin K antagonist.

## Ischemic events (MACCE)

The incidence of MACCE was numerically higher in TAT group, although not statistically significant (19.8% vs.12.5%, p=0.366), while it was comparable between VKA and DOAC group (17% vs.17%, p=1.000). MACCE were more common in patients presenting with ACS at enrollment (23.5% vs 2.3%, p=0.002). The laboratory variables statistically different between patients who experienced MACCE and others are listed in table 4.1.

**Table 4.1**. Difference in the distribution of variables according to the incidence of MACCE, verified with Mann-Whitney or T-test student, as appropriate.

| | MACCE (n=25) | No MACCE (n=122) | P value |
|---|---|---|---|
| **TG time-to peak [min],** median (IQR) | 10.2 (7.5-11.9) | 12.0 (8.5-16.2) | 0.023 |
| **D-Dimer [ng/mL FEU],** median (IQR) | 1412 (872-2878) | 882 (496-1618) | 0.021 |
| **$P2Y_{12}$ dependent platelet reactivity [PRU]** (mean±SD) | 111±23 | 80±7.3 | 0.004 |
| **VWF** (mean±SD) | 292±189 | 220±56 | 0.147 |

Abbreviations: VWF: Von Willebrand factor; TG, thrombin generation.

All the variables that were associated with the incidence of MACCE at the univariate Cox regression analysis with a p value <0.100 were therefore included in the multivariable Cox regression analysis and shown in table 4.2. Von Willebrand factor and $P2Y_{12}$ dependent platelet reactivity levels resulted independent predictors of MACCE (HR 1.52, 95% CI 1.14 to 2.04, p=0.005 and HR 1.56, 95% C.I. 1.00 to 2.42, p=0.049, respectively).

**Table 4.2.** Univariate and multivariable analysis for the incidence of MACCE.

| | Univariate | | Multivariable | |
|---|---|---|---|---|
| **Variable** | **HR (95% C.I.)** | **P value** | **HR (95% C.I.)** | **P value** |
| **ACS** | 10 (1.35-74.24) | 0.024 | | |
| **LVEF** | 0.97 (0.94-1.00) | 0.082 | | |
| **$CHA_2DS_2VASc$ score** | 1.32 (0.98-1.80) | 0.073 | | |
| **eGFR*** | 0.67 (0.44-0.99) | 0.048 | | |
| **D-dimer*** | 1.45 (1.13-1.90) | 0.004 | | |
| **VWF*** | 1.63 (1.24-2.16) | 0.001 | 1.52 (1.14-2.04) | 0.005 |
| **$P2Y_{12}$ dependent platelet reactivity [PRU] *** | 1.72 (1.22-2.42) | 0.002 | 1.56 (1.00-2.42) | 0.049 |

*HR for each increase of 1SD. Abbreviations: VWF: Von Willebrand factor; ACS: acute coronary syndrome; eGFR: estimated glomerular filtration rate; LVEF: left ventricular ejection fraction; PRU: $P2Y_{12}$ reactivity unit.

More than half of ischemic events occurred in the first three months of therapy (14, 56%). The landmark analysis performed in this amount of time confirmed the association between MACCE and increasing levels of PRU, together with higher leukocyte count values (table 4.3). Moreover, the concomitant TXA2-dependent and $P2Y_{12}$-dependent high platelet response (HPR, defined as PRU levels>208 and ARU levels >550) resulted independently associated with the incidence of MACCE. This condition was observed in 15 patient (10.2%).

**Table 4.3.** Univariate and multivariable analysis for the incidence of MACCE in the first three months.

| | Univariate | | Multivariable | |
|---|---|---|---|---|
| **Variable** | **HR (95% C.I.)** | **P value** | **HR (95% C.I.)** | **P value** |
| **ACS** | 5.81 (0.76-44.60) | 0.089 | | |
| **MI at index hospitalization** | 6.0 (1.34-26.87) | 0.019 | | |
| **White bood cells*** | 1.66 (1.01-2.74) | 0.047 | 2.01 (1.01-3.99)[§]<br>2.63 (1.17-5.87)[+] | 0.046<br>0.019 |
| **VWF*** | 1.60 (1.15-2.20) | 0.005 | | |
| **D-dimer*** | 1.55 (1.14-2.10) | 0.005 | | |
| **PRU*** | 1.77 (1.20-2.60) | 0.004 | 1.65 (1.02-2.68) | 0.042 |
| **Dual antiplatelet drug "resistance"** | 3.99 (1.25-12.72) | 0.019 | 35.81 (4.80-267.18) | <0.001 |

§ model with PRU; + model with dual platelet "resistance"; *HR for every increase of 1SD. Abbreviations: VWF: Von Willebrand Factor; ACS: acute coronary syndrome; MI, myocardial infarction; PRU: $P2Y_{12}$ reactivity unit.

Since patients in DTA were not receiving aspirin (and therefore had ARU levels>550 by definition), we performed a Kaplan-Meier analysis considering only patients in TAT, confirming the association between dual antiplatelet drug resistance and MACCE occurrence (HR 7.65, 95%C.I. 1.58-37.04, p=0.012).
Figures 3.2 and 3.3 show 3-months survival curves free from MACCE, according to presence of $P2Y_{12}$-dependent platelet resistance (HR 3.54, 95% C.I. 1.19 to 10.58, p=0.015, figure 3.2) and dual platelet resistance in the whole population (HR 3.99, 95% C.I. 1.25 to 12.72, p=0.011, figure 3.3).

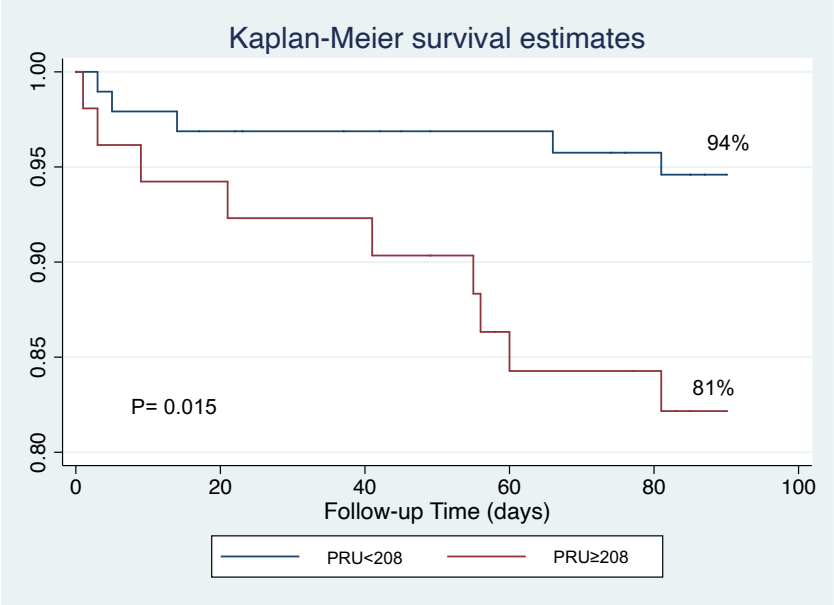


**Figure 3.2.** Kaplan-Meier curve showing MACCE free-survival according to presence of $P2Y_{12}$-dependent platelet resistance in the first 3 months (PRU≥208U).

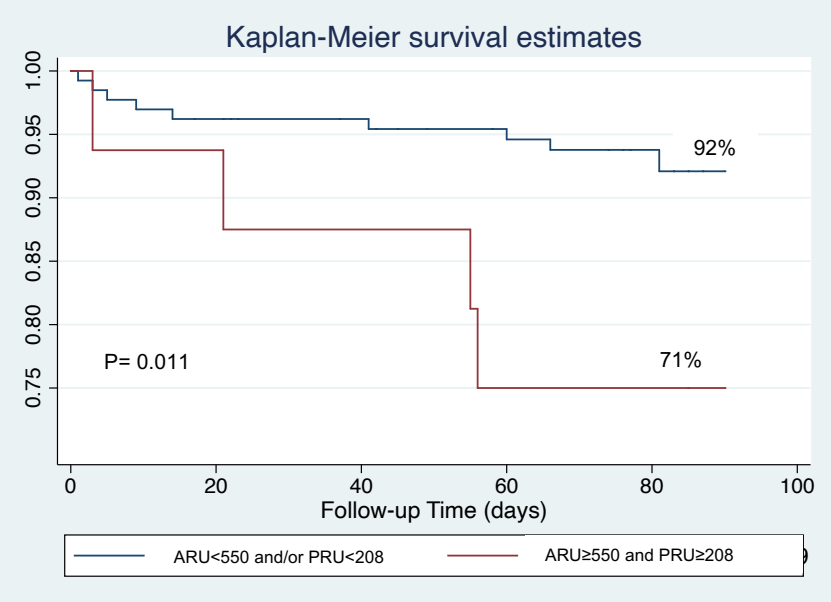



**Figure 3.3.** Kaplan-Meier curve showing MACCE free-survival according to dual antiplatelet drug "resistance" in the first 3 months (PRU≥208U and ARU≥550U).

A ROC curve was performed in order to identify a value of PRU to be considered as a cut-off in the present population, in which antiplatelet and anticoagulant therapy coexist. The Area Under Curve was 0.651 and the value identified was 186 U (figure 3.3). Kaplan-Meier curve confirmed a significant association between MACCE and this cut-off (HR 7.61, 95% C.I. 1.70 to 34.01, p=0.002, figure 3.5).

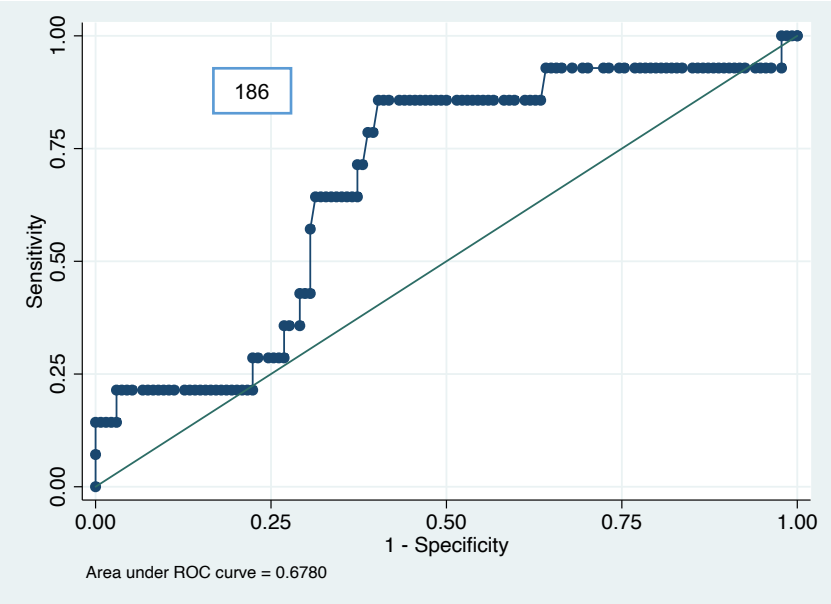



**Figure 3.4.** ROC curve of PRU values according to MACCE incidence.

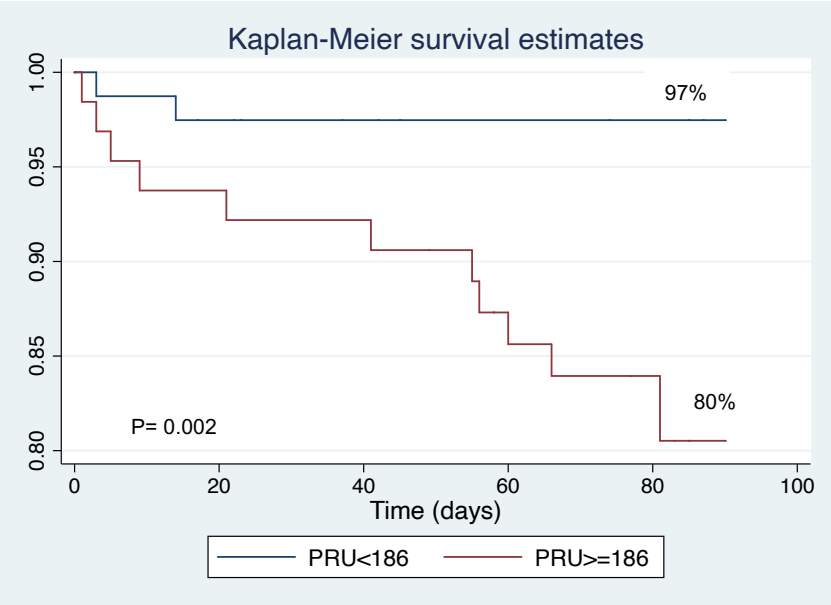



**Figure 3.5.** Kaplan-Meier curve showing MACCE free-survival according to PRU levels in the first 3 months (PRU cut-off 186U).

## Hemorrhagic events

Bleeding events were comparable between TAT and DAT group and between DOAC and VKA group. In major/clinically relevant bleeding group, a significantly lower level of ADAMTS-13 (60.43±25.41 vs 73.1±22.95, p=0.030) and a higher mean ORAC ratio (1.12±0.38 vs. 0.96±0.49, p=0.035) were observed (table 5.1).

**Table 5.1**. Difference in the distribution of variables according to the incidence of major/clinically relevant bleeding, verified with Mann-Whitney or T-test student, as appropriate.

| | Major/clinically relevant bleeding (n=19) | Minor/no bleeding (n=128) | P value |
|---|---|---|---|
| **ADAMTS-13** (mean±SD) | 60.43±25.41 | 73.1±22.95 | 0.030 |
| **VWF/ADAMTS-13,** median (IQR) | 3.70 (3.26-4.77) | 2.95 (2.23-3.94) | 0.070 |
| **ORAC ratio** (mean±SD) | 1.12±0.38 | 0.96±0.49 | 0.035 |
| **ETP with thrombomodulin** (mean±SD) | 390 (224-747) | 681 (408-1211) | 0.075 |

Abbreviations: VWF, Von Willebrand factor; ETP, endogenous thrombin potential.

Table 5.2 shows the variables associated with an increased risk of major/clinically relevant bleeding. A lower oxidative stress index (expressed as high values of ORAC ratio) resulted to be the only significant independent bleeding predictor (HR 7.77, 95% CI 1.61-37.38, p=0.011).

**Table 5.2.** Univariate and multivariable analysis for the incidence of major/clinically relevant bleeding.

| | Univariate | | Multivariable | |
|---|---|---|---|---|
| **Variable** | **HR (95% C.I.)** | **P value** | **HR (95% C.I.)** | **P value** |
| **Active smoke** | 3.39 (1.22-9.42) | 0.019 | | |
| **Previous MI** | 0.29 (0.08-1.00) | 0.050 | 0.15 (0.17-1.25) | 0.079 |
| **MI at index hospitalization** | 2.90 (1.04-8.06) | 0.041 | | |
| **Multivessel disease** | 4.31 (0.99-18.65) | 0.051 | | |
| **LM disease** | 3.12 (1.23-7.92) | 0.016 | 3.20 (0.98-10.42) | 0.053 |
| **INR*** | 2.26 (1.27-4.05) | 0.006 | | |
| **D-dimer*** | 1.32 (0.97-1.81) | 0.080 | | |
| **ADAMTS-13*** | 0.63 (0.41-0.97) | 0.036 | 0.61 (0.36-1.01) | 0.056 |
| **ORAC ratio*** | 2.11 (0.98-4.54) | 0.056 | 7.77 (1.61-37.38) | 0.011 |

*HR for every increase of 1SD. Abbreviations: INR, international normalized ratio, MI, myocardial infarction; LM, left main.

The ROC curve elaborated according to ORAC ratio values (figure 4.1), showed moderate accuracy (AUC 0.726). The Kaplan-Meier curve created with the identified cut-off of 0.905 (figure 4.2), confirmed a superior risk of bleeding in patients with low oxidative stress.

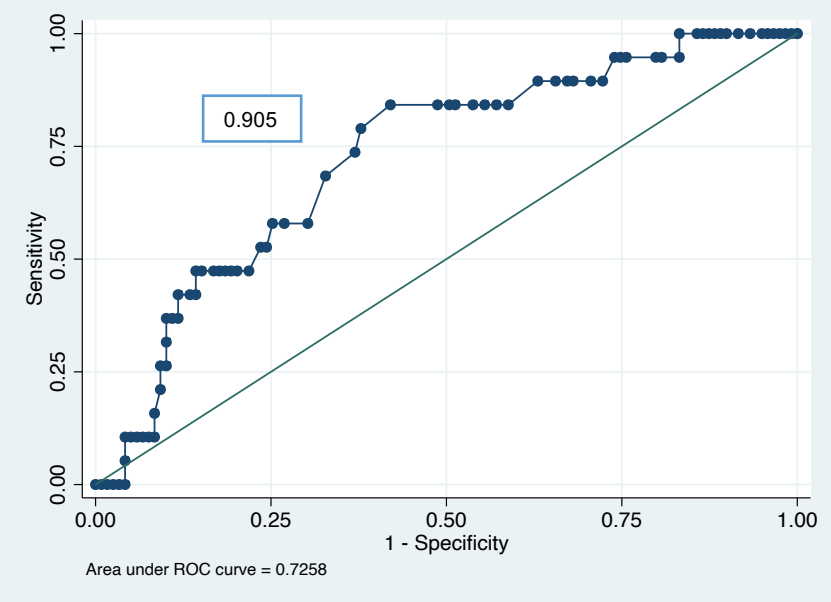



**Figure 4.1** ROC curve of ORAC ratio values according to major/clinically relevant bleeding incidence.

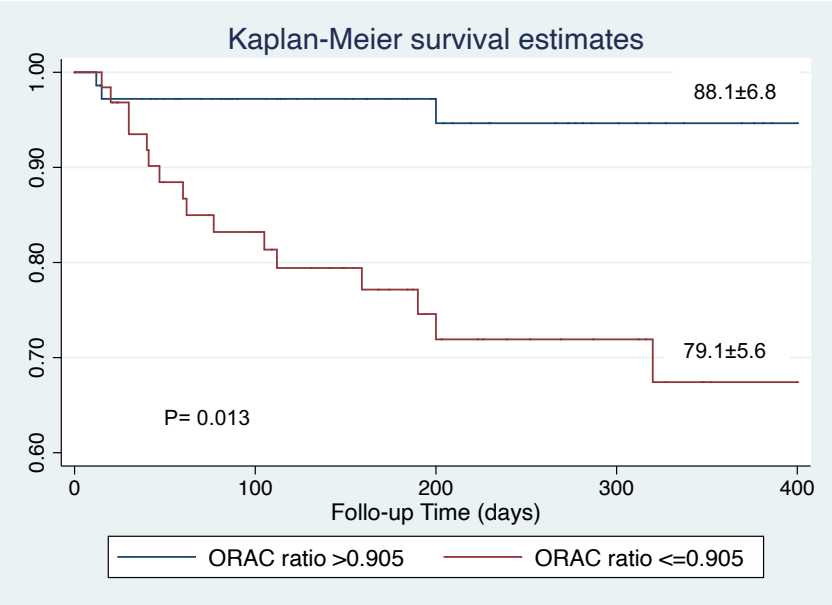



**Figure 4.2.** Kaplan-Meier curve showing major/clinically relevant bleeding free-survival according to ORAC ratio levels (cut-off 0.905).

Globally the presence of an imbalance between antioxidative and oxidative factors (ORAS/TBARS ratio) was significantly associated with the incidence of bleeding events, as represented in figure 4.3, where the curve was performed considering increasing tertiles of ORAC/TBARS (cutoff 20.24 and 32.75).

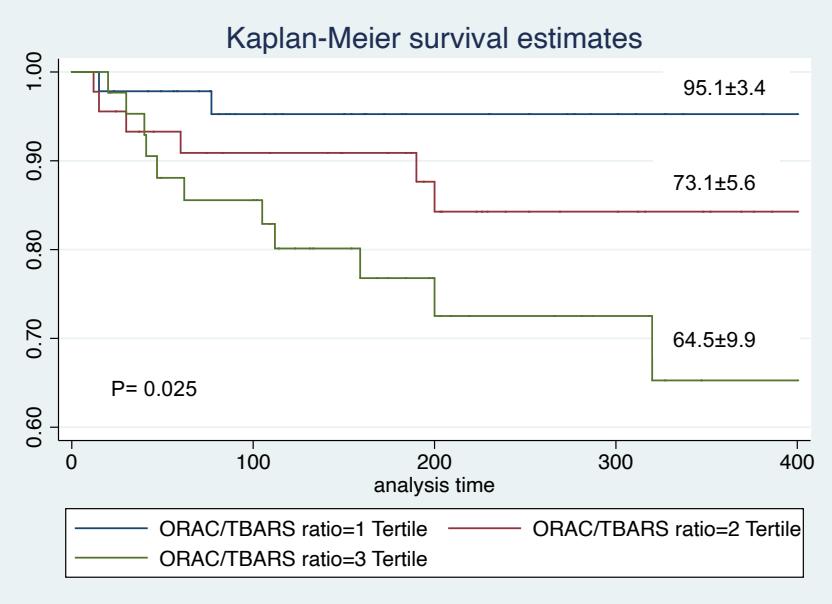



**Figura 4.3.** Kaplan-Meier curve showing bleeding free-survival according to ORAC/TBARS ratio tertiles.

Finally, since most patient on DOAC were taking dabigatran (n=41, 41%), we created a ROC curve to find plasmatic level cut-off value that could be associated with bleeding risk (figure 4.4). This curve showed good accuracy (AUC 0.88), and the Kaplan-Meier curve demonstrated that patients on dabigatran with

plasmatic concentrations higher than 101ng/ml were significantly more predisposed to hemorrhagic events (figure 4.5).

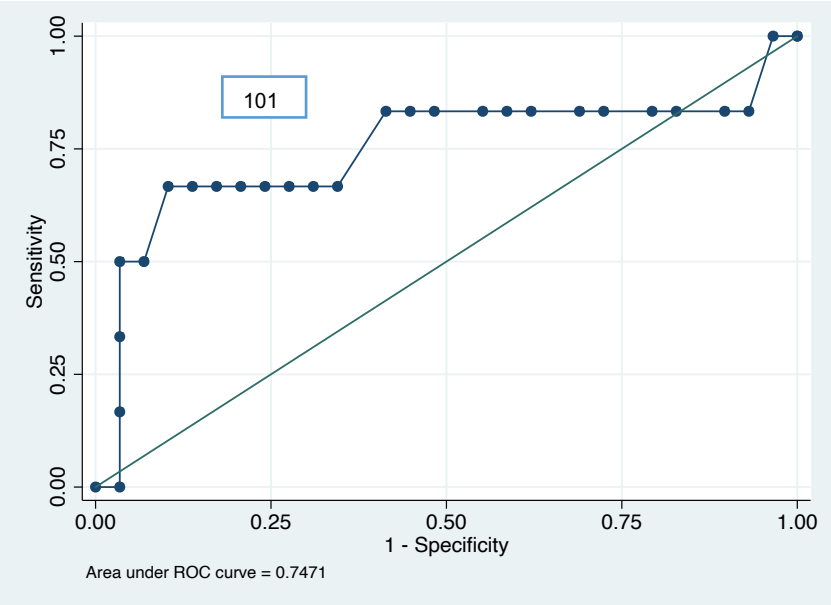



**Figure 4.4.** ROC curve of dabigatran plasmatic levels according to major/clinically relevant bleeding incidence.

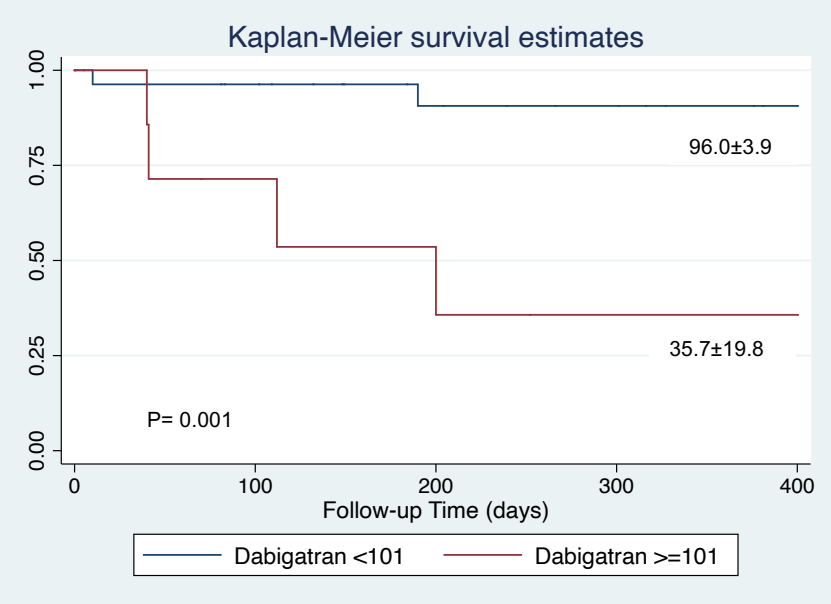



**Figure 4.5.** Kaplan-Meier curve showing bleeding free-survival according to Dabigatran plasmatic levels (cut-off 101 ng/ml).

## Mortality

The rate of all-cause mortality was not different between patient in TAT and those in DAT, while a trend towards higher risk of death was seen in patients in VKA (20% vs 9%, p= 0.063), especially in the first three months (figure 5.1).

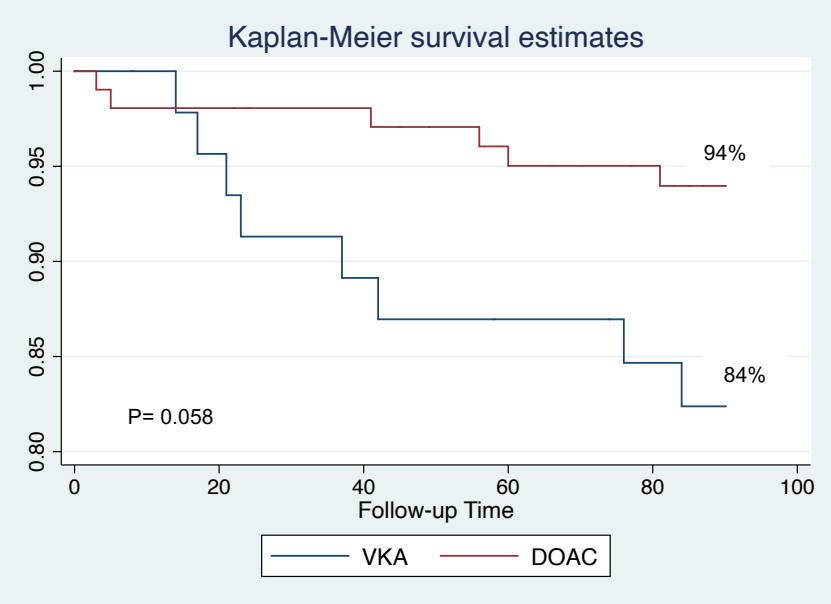



**Figure 5.1.** Kaplan-Meier curve showing survival according to type of anticoagulant therapy.

A higher number of deaths was also seen in females (22.9% vs. 7.2%, p=0.007), in patients with known peripheral artery disease (23.4% vs. 8%, p=0.013) and in those who presented with a myocardial infarction at enrollment (18.4% vs. 5.8%, p=0.021). Other continuous variables that had a significantly different distribution according to the incidence of death are showed in table 6.1.

**Table 6.1**. Difference in the distribution of variables according to the incidence all-cause death, verified with Mann-Whitney or T-test student, as appropriate.

| | Non-survivors (n=18) | Survivors (n=129) | P value |
|---|---|---|---|
| **Age [years],** mean±SD | 82±6 | 77±8 | 0.003 |
| **$CHA_2DS_2VASc$ score,** mean±SD | 5.4±1.5 | 4.7±1.2 | 0.019 |
| **HASBLED score,** mean±SD | 2.7±0.8 | 2.4±0.7 | 0.068 |
| **Red blood cells count,** mean±SD | 3.73±0.46 | 4.13±0.68 | 0.011 |
| **eGFR dim [ml/min},** mean±SD | 38±19 | 53±24 | 0.014 |
| **aPTT [sec],** median (IQR) | 28.9 (25.9-36.6) | 32.2 (29.9-36.5) | 0.039 |
| **D-Dimer,** median (IQR) | 1658 (1053-2462) | 895 (492-1535) | 0.002 |
| **ADAMTS-13,** mean±SD | 59.67±19.16 | 72.93±23.83 | 0.006 |
| **VWF/ADAMTS-13,** median (IQR) | 4.35 (3.22-7.00) | 2.97 (2.27-3.91) | 0.030 |

| | | | |
|---|---|---|---|
| **TBARS,** median (IQR) | 0.84 (0.53-1.12) | 0.62 (0.37-0.77) | 0.033 |

Abbreviations: eGFR, estimated glomerular filtration rate; VWF, Von Willebrand factor, aPTT activated partial thromboplastin time.

Table 6.2 displays univariate and multivariable analysis for all-cause mortality. Female gender, increasing age, higher white blood cells count level and oxidative stress proved independent predictors of death.

**Table 6.2.** Univariate and multivariable analysis for the incidence of all-cause death.

| | Univariate | | Multivariable | |
|---|---|---|---|---|
| Variable | HR (95% C.I.) | P value | HR (95% C.I.) | P value |
| Age* | 2.48 (1.34-4.60) | 0.004 | 3.52 (1.56-7.93) | 0.002 |
| Male sex | 0.27 (0.11-0.70) | 0.007 | 0.26 (0.07-0.90) | 0.033 |
| Weight | 0.64 (0.38-1.07) | 0.090 | | |
| PAD | 3.2 (1.23-8.23) | 0.017 | | |
| MI at index hospitalization | 3.46 (1.14-10.5) | 0.029 | | |
| DOAC vs. VKA | 0.44 (0.18-1.11) | 0.082 | | |
| $CHA_2DS_2VASc$ score | 1.59 (1.11-2.29) | 0.012 | | |
| HASBLED score | 1.76 (0.99-3.13) | 0.054 | | |
| White blood cells count* | 1.63 (1.03-2.58) | 0.038 | 2.75 (1.49-5.08) | 0.001 |
| Red blood cells count * | 0.52 (0.31-0.87) | 0.014 | 0.41 (0.22-0.76) | 0.005 |
| eGFR* | 0.5 (0.30.0.84) | 0.010 | | |
| D-dimer | 1.40 (1.02-1.90) | 0.036 | | |
| ADAMTS-13* | 0.63 (0.41-0.99) | 0.046 | | |
| VWF* | 1.5 (1.2-2.0) | 0.003 | | |
| TBARS* | 2.79 (0.99-7.82) | 0.051 | 8.31 (2.23-30.94) | 0.002 |

Abbreviations: PAD, peripheral artery disease; MI, myocardial infarction; eGFR, estimated glomerular filtration rate; VWF, Von Willebrand facto.,

The ROC curve made on TBARS values, the variable showing the strongest association with the risk of death, led to identify the cut-off value of 0.805 (figure 5.2). The Kaplan-Meier curve confirmed the strong association between high level of oxidative stress and all-cause mortality (figure 5.3).

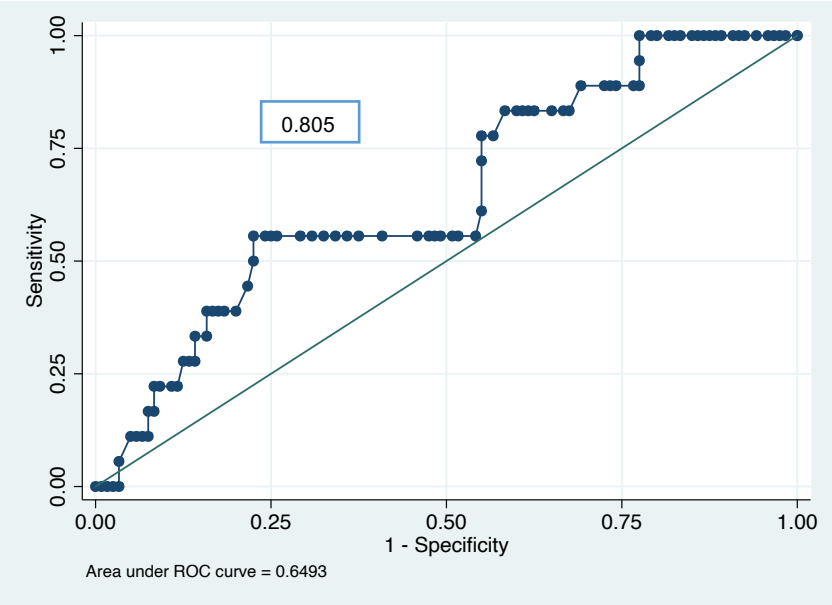



**Figure 5.2** ROC curve of TBARS values according to all-cause mortality risk.

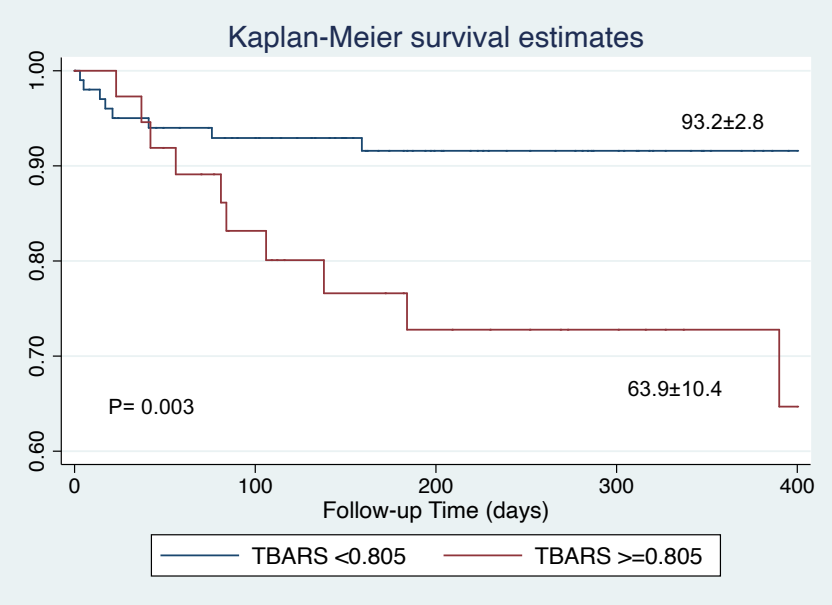



**Figure 5.3.** Kaplan-Meier curve showing survival according to TBARS value, cutoff 0.805.

# DISCUSSION

The present prospective registry was meant to find potential predictors of hemorrhagic or ischemic events as well as death in a high-risk population, discharged with antiplatelet and anticoagulant therapy in combination.

The results can be summarized in the following points:

- In a real-world unselected population, the choice of the more appropriate antithrombotic pattern was various, as expected, but TAT was still the most prescribed and it is carried on for at least one month after discharge in most subjects.
- In DAT clopidogrel was far the preferred antiplatelet agent.
- The decision on the type of antithrombotic pattern in favor of TAT was mainly driven by coronary complexity, while $CHA_2DS_2$-VASc e HAS-BLED score did not differ between TAT or DAT group.
- The usage of DOAC was associated with a lower thrombin inhibition as compared with those observed with VKA; besides in DOAC group a lower oxidative stress was found.
- Ischemic events were even more common that clinically relevant bleeding, despite combination therapy.
- No significant differences were found in terms of hemorrhagic or ischemic events in patients in TAT or DAT or with different anticoagulants. Patients in VKA showed a trend toward higher mortality rate.
- $P2Y_{12}$ and combined $P2Y_{12}$ and TxA2 dependent HPR were independently associated with the risk of ischemic events, especially in the first three months after PCI.
- VWF resulted independent predictor of MACCE.
- Higher levels of anti -II activity on dabigatran were associated with hemorrhagic risk.
- Higher inflammatory levels at discharge were independent predictors of mortality and ischemic events.
- High levels of oxidative stress were associated with an increased risk of all-cause death, low levels were found in patients experiencing a hemorrhagic event.

## 1. Choice of antithrombotic pattern

The first consideration that can be made is about clinicians' choice in antithrombotic pattern at discharge. The majority of patients were prescribed with TAT, which was carried on for at least 1 month in most patients. This choice was mostly driven by the presence of a more severe coronary scenario, characterized by acute presentation but also by multivessel disease and more complex PCI. These data are in line with what it has been found in all the trials comparing TAT and DAT, where a higher risk of thrombosis was observed in DAT patients.[72,74,76,77,95] For this reason, 2018 European guidelines on coronary revascularization recommended a minimum of 1 month of TAT in patients perceived at high ischemic risk.[51] More recently, even in patient at high bleeding risk, both European and American guidelines suggests to

keep TAT at least for the periprocedural time (1 to 7 days after PCI), in order to minimize stent thrombosis risk;[23,80] this is supported by the fact that in the AUGUSTUS trial, patients were enrolled at a median of 6 days from ACS and/or PCI, which suggests that most patients in the trial had at least short-term aspirin use before randomization.[76]

TAT was also preferred in patients with worse renal function. Chronic kidney disease (CKD) is a well know ischemic risk factor both in CAD and AF patients,[96–98] and European guidelines recommend to consider it in the management of antithrombotic therapy, despite not included in validated score as $CHA_2DS_2VASc$ or DAPT.[23,56] On the other side, bleeding risk in patients with renal disfunction treated with antithrombotic agents is high, especially in end-stage renal disease (ESRD)[99] and it is also included in hemorrhagic risk score such as HAS-BLED.[25] Interestingly, all patients with ESRD in the present registry were discharged with TAT, suggesting that ischemic risk is perceived as predominant in this setting. Actually, these subjects had a complex coronary profile, with acute presentation and multivessel disease with LM involvement in almost all cases, often accompanied by severe PAD. Besides, a lower mean eGFR characterized also patients receiving VKA, probably because of DOAC's contraindication in subjects with severe renal disfunction. This data may explain the higher mean HAS-BLED score found in patients in VKA.

On the other side, DOACs were preferred in older patients and in those with a previous hemorrhagic event, especially in TAT. This reflects the well-known benefit of DOACs in the reduction of bleeding risk with respect to VKA.[37] One limitation of the present analysis is the lack of data about frailty, which could have conditioned clinicians in the choice of antithrombotic pattern. $CHA_2DS_2$-VASc and HAS-BLED did not seem to guide this decision.

Another consideration can be drawn from TAT extension. Globally the median time of TAT was 3 months. During the course of the study, clinicians' attitude has changed according to guidelines updates. In fact, while previous European guidelines contemplate the possibility of keeping TAT up to 6 months after stenting,[56] the last recommendations, as already mentioned, reduced TAT window at a maximum of one month.[23,80] However, these data do not necessarily reflect a weighted decision: in several cases antithrombotic therapy was maintained by the patient until the subsequent medical examination, despite a different recommendation in the discharge letter. This aspect suggests that the communication with the patient should be reinforced, especially in older subjects and when a considerable number of pills is prescribed.

## 2. Laboratory analyses

Laboratory parameters analyses showed a significantly higher residual ETP in patients receiving DOAC compared to those in VKA. This data has been already described and could reflect a more stable anticoagulation profile with VKA, associated to their longer half-life.[100] Besides, a Dutch study demonstrated a paradoxical increase of peak and ETP in patients treated with dabigatran, compared to antiXa drugs, secondary to CAT-algorithm, that uses as calibrator the α2-macroglobulin-thrombin complex,

without calculating dabigatran inhibitor effect.[101] However, in the present analysis the difference observed persisted even after excluding patients in dabigatran, suggesting the presence of a class effect.

# 3. Follow-up events

Unexpectedly, the incidence of ischemic events was at least comparable to that of bleeding ones. In fact, in this clinical setting, the most relevant issue is the extremely high hemorrhagic risk associated with the combination of both antiplatelet and anticoagulant agents. The results of the present analysis highlight the need of not undervaluing thrombotic risk, especially early after stenting. Actually, most of these events occurred in the first three months after enrollment, even if the risk persists across the subsequent follow-up. It is worth considering that this was a very high-risk population: mean age was 78 years old, nearly one subject out of two had undergone a coronary revascularization before index hospitalization, which occurred for ACS in 70% of cases; besides the prevalence of other conventional risk factors was not negligible. However, this condition should be considered as a strength of this registry, since it represents a picture of a real-world unselected population, not necessary resembling that enrolled in RCT, but often encountered in clinical practice. Moreover, these data confirm that even if bleeding is the most feared event, elderly carries a significant high ischemic risk, as already demonstrated,[102,103] and therefore adequate antithrombotic therapy should not be precluded in older patients for age-related reasons only.

In contrast to what expected, neither the type of antithrombotic pattern or of anticoagulant agent were associated with a statistical different incidence of ischemic or bleeding events. This may be interpreted as a consequence of the non-randomized nature of this registry and of the limited sample seize. Notwithstanding this aspect, several independent predictors of ischemic/hemorrhagic events and mortality emerged from the analysis.

## Platelet inhibition

The use of platelet function tests (PFTs) to tailor antiplatelet drugs in cardiovascular patients has been debated for the last ten years. In spite of numerous studies and meta-analyses confirming the associations between HPR and cardiovascular outcomes among patients treated with $P2Y_{12}$ inhibitors,[104–106] RCT failed to demonstrate any benefit of a PFT-guided strategy compared to standard care.[107–109] This can be partially explained considering that HPR showed prognostically relevant in ACS patients,[110] and the latest advances in the treatment of these patients, with potent $P2Y_{12}$ inhibitors and new-generation DES have substantially closed the discussion about antiplatelet drug escalation based on PFTs. Nevertheless, TROPICAL-ACS trial demonstrated that PFT may still have a role in de-escalation (i.e., reducing bleeding risk by replacing prasugrel or ticagrelor with clopidogrel). In this trial, 2610 ACS patients were randomized to a conventional DAPT arm with prasugrel or a strategy guided by a PFT. In the latter arm, participants were treated with prasugrel for 1 week, then clopidogrel for 1 week at which time they underwent PFT. Patients with documentation of HPR (n = 511, 39%) were switched back to prasugrel. The study showed that the strategy guided by PFT was not inferior to standard DAPT, since there were no significant differences between the

arms regarding either ischemic or bleeding events.[111] For this reason in cases of ACS, the 2018 ESC guidelines on revascularization suggest that de-escalation, but not escalation, of $P2Y_{12}$ inhibitors guided by a PFT may be considered, with a class IIb grading.[51] A recent American consensus documents claimed against the routine use of PFT to escalate treatment in patients with HPR on clopidogrel, while stating that its very selective and optional use is reasonable to consider in specific clinical scenarios in which adequate platelet inhibition is crucial (e.g., LM PCI, last patent vessel PCI, complex lesions, 2-stent bifurcation treatment, prior stent thrombosis) in patients not at excessive risk for bleeding.[112]

In the contest of combined antithrombotic therapy, the evidence on PFT application is still limited. A recently published registry enrolling patients with ACS and an indication for OAC failed to demonstrated any significant relationship between HPR and MACCE.[113]

In our registry HPR was a potent independent predictor of ischemic events, especially in the first three months: patients with PRU levels above the cut-off of 208 at Verify-now assay showed a significantly higher risk of MACCE, as we already demonstrated in ACS patient in DAPT.[114,115] The prevalence of HPR was consistent with other aforementioned trials and the first published study assessing this issue (n=50, 34%).[116] Since this cut-off was validated in patients in DAPT, we hypothesized that in this setting,-where antiplatelets are always combined with anticoagulant agents and aspirin is omitted in several cases,- a different value could better discriminate subjects at higher ischemic risk. Indeed, the cut-off of 186 U was more effective, suggesting that when DAPT is early discontinued a deeper $P2Y_{12}$ inhibition may be required, despite anticoagulation background.

Besides, dual HPR (both to aspirin and clopidogrel) was associated with extremely high risk of MACCE. This condition, already described in other contests,[117] characterized patient whose platelets are functionally not inhibited after coronary stenting, justifying the particularly high ischemic risk.

We believe that in the setting of combined antithrombotic therapy, PFT could still have a pivotal role in guiding antithrombotic strategy, since we are often dealing with ACS patients receiving mostly clopidogrel alone or DAPT for a brief amount of time. Indeed, the aforementioned American consensus document included this situation in the proposed indications for testing.[112] Further studies assessing if a PFT-guided strategy affects prognosis are warranted. In fact, even if prasugrel and ticagrelor are contraindicated in TAT, some limited evidence with ticagrelor in DAT came from RCT, and 2019 ESC guidelines considered its prescription as an alternative to TAT in patients with a moderate or high risk of stent thrombosis (class IIb, C).[53]

## Von Willebrand factor, ADAMTS-13 and inflammation

Another interesting finding of the present study is the positive association between VWF levels and incidence of MACCE. During ACS, VWF multimers in the ultra-large forms are released by endothelial cells when stimulated by several factors (i.e thrombin and cytokines),[118,119] they bind the glycoprotein 1b-IX complex and aggregate platelets.[120] These large multimeric forms are rapidly cleaved by the metalloprotease ADAMTS-13 on the surface of the endothelium.[121] If ADAMTS-13 activity is reduced, the

ultra-large VWF accumulate and can promote platelet aggregation.[122] High plasma VWF levels or low ADAMTS 13 levels have been consistently associated with the risk of CVD, ACS and stroke.[123–127]

In a published paper of our group, we found that HPR was associated with lower ADAMTS-13 activity accompanied by elevated VWF levels independently of the platelet activation mechanism - thromboxane or ADP - in ACS patients on DAPT after PCI.[128] In the present analysis both VWF and PRU levels were independent predictors of MACCE, suggesting the strong association between these proteins and ischemic events.

More interestingly, we found that a higher value of WBC count was an independent predictor of both MACCE and all-cause mortality. The close link between CAD, ACS and inflammation has long been known, so much that atherosclerosis itself has been defined as a chronic vascular inflammatory process.[129] Leucocytes are found in unstable plaques and coronary thrombi,[130,131] and ACS are characterized by high levels of IL-6, a marker of inflammation which has been found to be related with clinical outcomes.[132,133] In *"in vitro"* studies a decrease in ADAMTS-13 activity was induced by IL-6,[134] and VWF is actually considered an acute phase protein.[135] Besides, pro-inflammatory molecules promote the activation and migration of leukocytes to sites of vascular injury, where activated platelets coaggregate with them and after their adherence to the vascular wall, provide a sticky surface to recruit other leukocytes on the vessel wall.[136] Therefore, the higher leucocyte count we demonstrated in patients experiencing MACCE or death could be considered a proxy of an enhanced inflammation background that, in turn, can promote CV risk also through the participation of VWF. Finally, to come full circle, we already proved that ACS patients with pro-inflammatory cytokines not compensated by anti-inflammatory cytokines have higher risk of HPR by both AA and ADP.[128,137] A next step of our analysis would be the measurements of cytokines levels to search for other more close associations in this field.

## DOAC plasmatic concentrations

It is well-known that DOACs do not require monitoring of coagulation, since they were tested and validated without dose adjustments based on plasma level measurements. Moreover, no studies have investigated if measurement of drug levels and dose adjustment based on laboratory parameters improve the overall benefit of DOACs during long-term treatment. However, 2021 EHRA consensus document on the use of DOAC in patients with AF suggests that this assessment could be performed in rare situations, such as bleeding, urgent or certain elective procedures, suspected overdose, acute stroke, extremes of bodyweight or severely impaired renal function.[138] The range of expected plasma levels of DOACs are pretty wide: actually almost all of the patients enrolled in our registry displays in range-values of dabigatran concentration. However, we demonstrated a correlation between plasmatic level of dabigatran and hemorrhagic events. A similar result has already been observed in a Swedish study, where dabigatran plasma levels was assessed in 44 AF patients, founding significantly higher average trough concentrations in patients with minor bleeding (93 ± 36 versus 72 ± 62 μg/L, p = 0.02).[139] Unfortunately, in our study, this assessment could not be performed with other DOACs because of small numbers of treated patients.

Even in the knowledge that prospective RCT outcome data still do not exist to support such a concentration-guided strategy, guidelines contemplate a certain dosage tailoring beyond classic criteria. In fact, as a result of PIONEER and REDUAL-PCI trials, 2020 AF ESC guidelines suggest that in patients at high bleeding risk (HAS-BLED≥3), dabigatran 110 mg b.i.d. or rivaroxaban 15 mg should be considered in preference to dabigatran 150 mg b.i.d. and rivaroxaban 20 mg for the duration of concomitant single or DAPT, to mitigate bleeding risk.[23] Of course, the small sample size of the present, non-randomized registry does not allow to draw conclusions; however it confirms that, such in the case of platelet reactivity, assessing individual drug response could represent a useful aid in the tailoring-clinical decision making. Other analysis with larger sample size could better inform on this topic.

## Oxidative stress balance

A novel intriguing datum of the present analysis is the association between low oxidative stress and bleeding events. While the opposite, that is increased risk of ischemic events in presence of high oxidative stress has been extensively assessed,[140,141] our finding has not been directly addressed so far. Some evidence came from RCT developed in order to evaluate if the antioxidant effect of vitamin E could prevent ischemic stroke. Globally, the results were evaluated in the meta-analysis of Schürks et al,[142] which included 118765 participants, and suggested that vitamin E supplementation increases the risk for hemorrhagic stroke by 22%, but reduces the risk for ischemic stroke by 10%. In that contest this effect was interpreted as consequence of a possible interaction between vitamin E and antiplatelet/anticoagulant pathways. In our case series lower oxidative stress seem to be *per se* the strongest potent independent predictor of bleeding; besides, the cutoff found with ROC was surprisingly the same adopted in our laboratory to discriminate low oxidative stress levels. Further examinations are needed to identify possible pathophysiologic mechanisms involved and to confirm the clinical relevance of this finding.

On the other side we observed a strong association between high TBARS level and mortality. Age-accelerated vascular injury is commonly considered to result from increased oxidative stress.[143] In these conditions, aging is associated with immunosenescence and accompanied by a chronic inflammatory state which contributes to atherosclerosis and CVD.[144] Besides, it has been demonstrated that metabolic abnormalities as a result of aging cause platelet hyperaggregability involving enhanced intraplatelet reactive oxygen species (ROS) production and decreased nitric oxide bioavailability.[145,146]

Interestingly, we found that DOAC therapy was associated with lower oxidative stress levels as compared to VKA, a novel finding to our knowledge. A possible mechanism involved in this association might be the anti-inflammatory activity of DOAC observed in other scenarios, even if not prooved.[147] Moreover, the lower mortality rate observed with DOAC in other trials and observational studies[148,149] was also confirmed in the present registry, even if the statistical significance was not reached. It could be speculated that this benefit may be somehow related to the reduced oxidative stress associated with DOAC therapy. On the other side, the substantial reduction of hemorrhagic risk associated with DOAC contradicts the link between low oxidative stress and bleeding we found. Other evidence is needed to better clarify this topic.

# 4. Limitations

Several limitations of this analysis should be acknowledged.

1. Non-randomized design may have produced selection bias; however, this registry was born to evaluate a real-world population as discharged from hospital.
2. Sample size is limited. However, since the latest guidelines recommendations release, antithrombotic pattern has become more homogeneous, making impossible to perform any comparison between TAT and DAT.
3. The follow-up was mostly carried on by telephone, which could have led to reduced accuracy in the evaluation of therapy and events.
4. Antithrombotic therapy is of course dynamic during follow-up; the clinical and laboratory picture obtained at enrollment not necessary reflects the one present when an adverse event occurs. However, in clinical practice hospitalization for the index event is the crucial time when most decisions about patient's subsequent therapy are taken. For this reason, identifying an independent predictor at this point is of paramount importance to guide clinical decision-making.

# 5. Conclusions

The present analysis pointed out some laboratory markers of ischemic and hemorrhagic events in a high-risk population on dual or triple antithrombotic therapy. The setting of PCI performed in subjects with atrial fibrillation represents an ideal field of application of tailored medicine, since different type of drugs and duration of therapy can be combined in a myriad of possible strategies that need to be personalized according to patient's profile. The opportunity to add laboratory markers of drug response to classical risk score, could better raffinate the clinical decision-making.

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