Fighting against atherosclerotic disease: From the endothelium to invasive cardiology

PhD Thesis

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General introduction and outline of the thesis

Part I. In vitro strategies to improve endothelial function and response to ischemia

Cardiovascular diseases (CVD) are a heterogeneous group of disorders of the heart and blood vessels including coronary heart disease, cerebrovascular disease, rheumatic heart disease and other conditions. CVD are still the most common cause of death worldwide. According to the actual estimate from World Health Organization 17.9 million people die each year from CVDs, an estimated 32% of all deaths worldwide (1). More than four out of five CVD deaths are due to heart attacks and strokes, and one third of these deaths occur prematurely in people under 70 years of age. While before the XX century infectious diseases and malnutrition were the most common causes and CVD were the cause of only 10% of all deaths, nowadays CVD account for about 30% of deaths worldwide, including nearly 40% in high-income countries and about 28% in low- and middle-income countries. Notably, >75% of CVD deaths occur in low- and middle-income countries (2).

The global rise in CVD derives from a significant transformation in the causes of morbidity and mortality during the twentieth and twenty-first centuries. This shift is driven by industrialization, urbanization, and deriving lifestyle changes and is taking place in every part of the world among all races, ethnic groups, and cultures, causing increased fat and caloric intake and decrease in physical activity leading to emergence and increased prevalence of arterial hypertension, smoking, overweight and diabetes mellitus, the classical risk factors for the development of atherosclerosis and, consequently, of myocardial infarctions, ischemic cardiomyopathies, cerebrovascular disease and peripheral obstructive disease (3).

The genesis of atherosclerosis in humans requires many years, usually decades (4). Growth and development of atherosclerotic plaques occur in a discontinuous way with periods of quiescence succeeded by phases of rapid evolution. After a prolonged “silent” period, atherosclerosis can become manifest. The clinical expressions may be chronic, such as in stable, effort-induced angina pectoris and/or intermittent claudication. Alternatively, it is also possible a dramatically acute clinical

course, with a myocardial infarction, a stroke, till to sudden cardiac death may be the first clinical appearance. The process leading to atherosclerotic plaque formation has several steps. “Fatty streak” represents the first lesion of atherosclerosis, arising from focal increases in the content of lipoproteins within the tunica intima (5). Lipoproteins in the extracellular space of the intima may undergo oxidative modifications, which trigger a local inflammatory response that mediates successive steps increasing the expression of adhesion molecules for leukocytes, which are recruited to the site of the nascent arterial lesion. Such leukocytes, and in particular, monocytes, in the evolving fatty streak exhibit augmented expression of receptors for modified lipoproteins (scavenger receptors). These mononuclear phagocytes ingest lipids and become foam cells, represented by a cytoplasm filled with lipid droplets (4,5). Notably, not all fatty streaks progress to the final atheroma. While the accumulation of lipid-laden macrophages is typical for fatty streaks, buildup of fibrous tissue formed by extracellular matrix typifies the more advanced atherosclerotic lesion. Smooth muscle cells synthesize the bone of the

extracellular matrix of the complex atherosclerotic lesion. Mononuclear phagocytes release growth factors and/or cytokines leading to stimulation and proliferation of smooth muscle cell and production of extracellular matrix. In the most advanced phases, foam cells may die by apoptosis. This death of mononuclear phagocytes results in the formation of a lipid-rich center, often called necrotic core in established atherosclerotic plaques (5). Other features of advanced plaque development are calcification and microvessels formation within the lesion. Plaque rupture by wall erosion and/or microvessel thrombosis or hemorrhages, with subsequent platelet and thrombin activations, are the mechanisms concurring to acute clinical events (5). A critical role in all this process is played by the endothelium. A deleterious alteration of endothelial physiology, universally known as endothelial dysfunction, represent a key step in the development of atherosclerosis and is critically involved in plaque progression and in atherosclerotic complications (6). Normal endothelium acts to mantain a normal vascular tone and blood fluidity, with expression of antinflammatory factors under normal

homeostatic conditions (6). Cardiovascular risk factors such as smoking, aging, hypercholesterolemia, hypertension and diabetes are all associated with alteration in endothelial function (7). Endothelial dysfunction plays a pivotal role in lesion formation by promotion of both the early and late mechanisms of atherosclerosis such as up-regulation of adhesion molecules, increased chemokine secretion and leukocyte adherence, increased cell permeability, enhanced low-density lipoprotein oxidation, platelet activation, cytokine elaboration, and vascular smooth muscle cell proliferation and migration (8,9). Endothelial dysfunction is also characterized by reduction of vasodilation, particularly nitric oxide (NO) (8,10), which moreover acts in the normal vessels by reducing expression of inflammatory and adhesion molecules that increase plaque vulnerability, and/or by an increase in endothelium-derived contracting factors (8,10)- as a consequence, endothelial dysfunction also plays a fundamental role in the pathogenesis of acute coronary syndromes and in the response to ischemic injury: in such context, a significant feature is the impairment of neo-angiogenesis, which should represent a sort of

compensative mechanism in the context of a vessel occlusion (6). Notably, oxidative stress may alter many endothelial functions, suggesting also an association between endothelial dysfunction and mitochondria (11). Diabetes mellitus impairs by multiple mechanisms endothelial homeostasis, including an increase in oxidative stress, oxidation of lipoproteins, phospholipids and proteins and enzymatic glycation of proteins and macromolecules and decreased insuline-induced NO-mediated vasodilation in insuline-resistant patients also for increased levels of vasoconstrictant agent such as endothelin (12).

A family of related mitochondrial protein kinase A anchor proteins (mitoAKAPs) is involved in the amplification within the cell of signal cues to mitochondria (13). MitoAKAPs are generated by alternative splicing of the *Akap1* gene and localize to mitochondria (13). The best-characterized *Akap1* product, the protein AKAP121, regulates mitochondrial structure and function and promotes cell survival under stressful conditions (14). AKAP121 undergoes rapid proteolytic degradation on hypoxia, which impairs mitochondrial function in heart and

isolated cardiomyocytes (14). *Akap1* also controls mechanistic target of rapamycin modulating cell growth (mTOR) (15). mTOR complex is a downstream effector of PI3K/Akt; notably, endothelial NO synthase enzyme phosphorylation by serine/threonine protein kinase Akt has an activation effect, with increased NO production (16). Whether *Akap1* might play a role in endothelial biology is not well characterized and will be the object of the study presented in Chapter 1.

Receptors for the Fc fragment (FcγR) of circulating immunoglobulins (Ig) have been identified in different cell types (17). Two FcγRs, i.e. FcγRI and FcγRIIB, are also expressed in endothelial cells (18). Data from animal studies state that IgG play a mechanistic role in atherosclerosis and diabetes through endothelial dysfunction and insulin resistance (19). Moreover, C Reactive protein (CRP) interacts with FcγRs on the endothelial cells (20). CRP is known to affect endothelial function, insulin action, and insulin-mediated glucose uptake (21). In mice studies, peripheral insulin resistance is linked to decreased endocytosis of serum insulin by the endothelial cells by hyposialylation of circulating IgG which results in FcγRIIB

stimulation (19). Effects of circulating IgG on the endothelium, altogether with regulation of peripheral blood flow and insulin sensitivity remain still unknown and will be the focus of the study presented in Chapter 2.

Part II. Clinical strategies to improve the outcome after percutaneous interventions

Together with development and refining of pharmacological strategies, the introduction of minimally invasive interventional procedures to diagnose and treat cardiovascular diseases, particularly cardiac and peripheral manifestations of atherosclerosis, has undoubtedly been the key for the progressive reduction in mortality and morbidity observed in the last forty decades for such pathologies, almost in most developed countries.

Egas Moniz performed the first cerebral angiography in 1927 at University of Lisbon (22); two years later, Werner Forssmann performed in Berlin the first human heart catheterization on himself by putting under local anesthesia a venous catheter from his own leg to right atrium (23). In 1958, Frank Mason

Sones Jr executed the first, unintended, coronary angiography (during an heart catheterization and an attempt to perform an aortography in a patient with rheumatic heart disease) (23); concerned for a fatal ventricular fibrillation, only a transient asystolia was observed; so the observation that dye contrast might be injected into coronary artery ostia led to subsequent refining of the technique for an adequate visualization of epicardial vessels and, eventually, of relative stenosis and occlusions (23). In 1966, the Argentine cardiac surgeon René Favaloro, performed the world's first coronary bypass surgery. Dr Favaloro called Sones "the most important contributor to modern cardiology" and said that without his work, "all our efforts in myocardial revascularization would have been fruitless". The shift from a diagnostic only technique to an interventional tool happens in 1964, when Charles Dotter, with his trainee Dr Melvin P. Judkins, performed the first percutaneous transluminal angioplasty (PTA) dilated a tight, localized stenosis of the superficial femoral artery (SFA) in an 82-year-old woman with painful leg ischemia and gangrene who refused leg amputation using a guide wire and coaxial

Teflon catheters (24). The artery remains patent until death of the patient from pneumonia two and a half years later (24). Finally, in 1974, Andreas Grüntzig performed the first balloon angioplasty on a severely diseases femoral superficial artery of a 67-years-old man by using a new single-lumen dilating catheter carrying a 4 mm inflatable balloon (24). While he continued to treat patients with peripheral vascular disease using new, double-lumen balloon catheters, he also began to explore other vascular territories to treat with his new technique. In 1977, after a failed first unsuccessful attempt in a patient with severe multivessel disease, and some intra-operative coronary angioplasties during cardiac surgery in San Francisco, Grüntzig made the first percutaneous coronary transluminal angioplasty in Zürich in a conscious 38-years old patient originally scheduled for bypass surgery because of an isolated LAD stenosis (24). After some initial concern, the procedure gains a wide and universal worldwide spread. Progressive advances in the technique and equipment led to increased safety and efficacy of the procedure. The development of metallic coronary stents (BMS) favored the

widespread adoption in routine clinical practice by eliminating the risk, not infrequent for balloon angioplasty, of recoiling and abrupt vessel closure as well as the need for standby emergent coronary artery bypass grafting (25). Nowadays, percutaneous coronary artery intervention constitutes the most commonly performed therapeutic procedure in cardiology. With progressive improvements in deliverability and reductions in acute thrombosis, the first limitation of BMS became in-stent restenosis, id est the recurrence of coronary stenosis within the stent due to neointimal hyperplasia resulting from smooth muscle cell proliferation and production of extracellular matrix, which led to the introduction of drug-eluting stents (DES) (25). DES delivers an antirestenotic drug to the arterial wall, thus decreasing rates of restenosis; unfortunately, first generation DES were associated with an increased risk of late (>1-year) thrombosis in comparison with BMS (25). Progressive improvement in technique led to development of new generation DES, with a broad range of refinements affecting the antiproliferative drug (from the old paclitaxel, widely used in the past, to the limus family of analogues of rapamycin) and

metallic stent platform (26). Morever, polymer coating of the stents have been associated with chronic inflammation, and, in some cases, with hypersensitivity reactions causing poor healing and delayed endothelialization of the device, potential triggers for late stent thrombosis (25). New devices have biocompatible permanent polymers or biodegradable polymers, till to complete removal of polymer coatings from stent surface. Bioresorbable vascular scaffolds (BRS) has represented a further evolution by affording a transient support to the coronary vessel for a variable period ranging with a progressive reabsorption and complete disappearance from the lumen of the vessel into a variable time. While the idea at basis was exceedingly enjoyable, avoiding permanent metallic cage into coronary vessels, especially for young patients, their fate into the clinical arena has not been brilliant (26). In Chapter III, there is a brief summary of the evidence regarding new generation DES and of BRS.

Improvement in pharmacological therapy and a widespread use of early reperfusion strategy in the acute phase are the main explanations for the observed fall in acute and long-term

mortality after myocardial infarction in the last twenty years (27,28). The prognostic advantage of immediate revascularization, in the form of primary percutaneous coronary intervention (PCI), in patients presenting with ST-elevation myocardial infarction (STEMI), and of an early and, in some cases, emergent revascularization in patients with non ST-elevation myocardial infarction (NSTEMI) has been clearly defined and stated in clinical Guidelines (29,30). However, mortality after myocardial infarction remains not negligible, being affected by several individual factors. Among them, the extension of coronary artery disease, particularly in the vessel not being "culprit" for the myocardial infarction, has been deeply focused. Such condition is known as multivessel coronary artery disease and occurs in about half of patients presenting with STEMI and undergoing primary PCI, and this affects both in-hospital and long-term clinical outcomes (29). Current evidence, deriving from recent randomized clinical trials, supports complete revascularization of residual significant non-culprit coronary artery lesions (29). Notably, an ideal tool for the assessment of which residual stenoses deserve

revascularization, as well as the best timing (in the acute phase, during the main hospitalization in a staged procedure or, perhaps, in a staged hospitalization) remain controversial. It has not been clearly defined if the definition of residual significant coronary stenosis in non culprit vessel should rely only on an angiographic evaluation (which might be subjected to inter-operator variability) or if use of functional invasive evaluation of ischemic potential by pressure-wire based techniques and/or intravascular imaging should have to be widespread in order to improve prognosis avoiding futile (and potentially harmful) procedures or the miss of treatment for relevant lesions. In the Chapter 4 all these themes are briefly summarized, while in the Chapter 5 the potential for prognostic evaluation purposes of a recently validated angiographic index (the ADDED Index), which takes into account the minimal lumen diameter of a coronary artery stenosis and the amount of jeopardized myocardium subtended and is able to accurately predict functional relevance of a coronary stenosis (31), has been evaluated in the setting of patients with residual coronary stenosis after culprit vessel PCI for STEMI, in comparison with

visually estimated angiographic significance. In the Appendix 1, preliminary data on comparison of ADDED Index versus the classic SYNTAX Score for risk stratification and prognostic evaluation of patients with incomplete coronary percutaneous revascularization after STEMI are shown.

Another detrimental factor affecting prognosis of patients with myocardial infarction undergoing percutaneous revascularization is bleeding. Bleeding events have been extensively associated with higher mortality rates in patients with acute coronary syndromes, due to perioperative events and successive visceral bleeds related to the unavoidable, "aggressive" antithrombotic therapy (32). In most cases, acetylsalicylic acid at "cardiac" dosages is combined with new potent platelet receptor P2Y12 inhibitors (such as prasugrel or ticagrelor) to prevent reinfarction and early stent thrombosis, a treatment known as dual antiplatelet therapy (DAPT). In some cases, an oral anticoagulant agent such as warfarin or the new direct oral inhibitors has to be associated (for instance, due to a concomitant atrial fibrillation, prosthesic heart valve or a left ventricle thrombosis) to a single antiplatelet or a dual

antiplatelet therapy (in the triple antithrombotic regimen). Such therapeutic regimens carry an intrinsic increase in bleeding risk. The observation that bleeding events significantly impairs prognosis for the risk of fatal events (such as intracranial hemorrhages, pericardial tamponade, or dramatic gastrointestinal acute bleeding) has focused the cardiovascular researchers to define strategies to limit potenzial hazards (such as the choice of radial access for coronary procedures and a tailored antithrombotic therapy taking into account not only the ischemic concerns but also the risk of bleeding) and to define risk scores and predictors of bleeding both for the acute phase and the post-hospitalization recovery (33). In the Chapter 6, the predictive prognostic role of a significant hemoglobin drop, also in the absence of an overt bleed, in patients subjected to percutaneous revascularization after acute coronary syndromes within the cohort of subjects enrolled in the MATRIX controlled randomized clinical trial is deeply described.

Percutaneous transluminal intervention represents the cornerstone for the improvement of atherosclerotic disease prognosis not only for the heart but also for peripheral arteries

affected by atherosclerotic obstruction. In the setting of lower limb arterial disease, percutaneous transluminal angioplasty has the potential to improve quality of life for patients with severe claudication and to preserve patients with critical limb ischemia from minor or major amputation (34). Femoropopliteal arteries represent the most frequently affected vessels from atherosclerotic obstructions. In most cases, surgical options are hampered by multiple comorbidities and worse long term prognosis (34). At the same time, while generally technically feasible in most cases, balloon angioplasty of femoropopliteal arteries has a significant rate of restenosis, mainly due to the muscular course of the artery which also compromises long term outcome of implanted stents: probably, this is the only anatomical setting where, nowadays, balloon angioplasty still represents the standard of care for percutaneous intervention, while stent deployment is considered only as a bail-out situation (acute recoil, long flow-limiting dissections) (34). The use of early generation drug-coated balloons has shown promising results in the prevention of restenosis in the femoropopliteal artery in randomized clinical trials (35); most of them, however,

enrolled patients with stable claudication and no critical limb ischemia; moreover, devices used in these trials have a huge number of technical issues such as inconsistent drug coating concentrations, significant drug loss prior to treatment, use of large paclitaxel particles which increases the risk of embolization, and excessive initial balloon-artery drug transfer rates resulting in early too high drug-in-tissue levels. In the Chapter 7, there are the results of a multicentre all-comers registry enrolling patients with femoropopliteal disease at any stage and without regard for type of lesion (de novo vs restenotis vs in stent restenosis) treated with the new Paclitaxel drug eluting balloon LEGFLOW® (Cardionovum), a new generation device covered with a homogenous and stable surface

coating using extremely small, non-visible Paclitaxel particles after one year follow up. In the Appendix 2, previously published interim results after six months follow up are shown.

Finally, in the Appendix 3, I have decided to enclose the results of a regional survey on trends in percutaneous interventions, performed by all cath-laboratories in Campania, during the first

COVID-19 outbreak in the spring of 2020 (to whom I collaborate in clinical data collection for my hospital, “San Giovanni Bosco” in Naples), while not strictly concerning the theme and the fil-rouge of this thesis. My hospital has been reconverted in the first six months in 2021, during the second and the third Italian outbreak of the pandemy, into a COVID-19 specialized hospital and I have worked in a dedicated cardiac coronary care unit and catheterization laboratory for people with documented SARS-COV2 infection, accepting patient from every hospital of the region. I will never be able to forget the fear of being infected, the sufferance in dyspnoic patients, the sense of impotence and sadness for experiencing, too often, patients’ death, alone, far from their loves. With eternal gratitude to science and clinical research for having given us the weapons to try to come out of this disgrace, I want to dedicate this little enclose to all the victims of the virus.

Original Article

Akap1 Regulates Vascular Function and Endothelial Cells Behavior

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Abstract—MitoAKAPs (mitochondrial A kinase anchoring proteins), encoded by the *Akap1* gene, regulate multiple cellular processes governing mitochondrial homeostasis and cell viability. Although mitochondrial alterations have been associated to endothelial dysfunction, the role of mitoAKAPs in the vasculature is currently unknown. To test this, postischemic neovascularization, vascular function, and arterial blood pressure were analyzed in *Akap1* knockout mice (*Akap1*$^{-/-}$) and their wild-type (wt) littermates. Primary cultures of aortic endothelial cells (ECs) were also obtained from *Akap1*$^{-/-}$ and wt mice, and ECs migration, proliferation, survival, and capillary-like network formation were analyzed under different experimental conditions. After femoral artery ligation, *Akap1*$^{-/-}$ mice displayed impaired blood flow and functional recovery, reduced skeletal muscle capillary density, and Akt phosphorylation compared with wt mice. In *Akap1*$^{-/-}$ ECs, a significant enhancement of hypoxia-induced mitophagy, mitochondrial dysfunction, reactive oxygen species production, and apoptosis were observed. Consistently, capillary-like network formation, migration, proliferation, and AKT phosphorylation were reduced in *Akap1*$^{-/-}$ ECs. Alterations in *Akap1*$^{-/-}$ ECs behavior were also confirmed in *Akap1*$^{-/-}$ mice, which exhibited a selective reduction in acetylcholine-induced vasorelaxation in mesenteric arteries and a mild but significant increase in arterial blood pressure levels compared with wt. Finally, overexpression of a constitutively active Akt mutant restored vascular reactivity and ECs function in *Akap1*$^{-/-}$ conditions. These results demonstrate the important role of mitoAKAPs in the modulation of multiple ECs functions in vivo and in vitro, suggesting that mitochondria-dependent regulation of ECs might represent a novel therapeutic approach in cardiovascular diseases characterized by endothelial dysfunction. (***Hypertension*****. 2018;71:00-00. DOI: 10.1161/HYPERTENSIONAHA.117.10185.)**
● **Online Data Supplement**

Key Words: angiogenesis ■ hypertension ■ ischemia ■ mitochondria ■ reactive oxygen species

Endothelial cells (ECs) dysfunction plays a central role in the pathogenesis of many cardiovascular disease, including atherosclerosis, coronary and peripheral artery disease, diabetes mellitus, hypertension, and heart failure.[1–4] A complex network of signaling pathways modulates ECs function under normal or pathological conditions.[5] Among these, endothelium-derived nitric oxide (NO) produced by the endothelial NO synthase (eNOS) is a fundamental determinant of endothelial and vascular homeostasis, regulating systemic blood pressure, vascular remodeling, and angiogenesis.[6] eNOS phosphorylation by serine/threonine protein kinase Akt mediates its activation, leading to increased NO production.[7] In addition, another serine/threonine protein kinase, the cAMP-dependent PKA (protein kinase A), has been shown to mediate multiple functions of ECs, regulating vascular development.[8]

Mitochondria are the major intracellular source of energy and reactive oxygen species (ROS) in several cells and tissues and contribute to the regulation of multiple cellular functions.[4,9,10] Accumulating evidence shows that oxidative stress may alter many endothelial functions,[5,11] suggesting an association between endothelial dysfunction and mitochondria.[9,12,13] Although oxidative phosphorylation plays a limited role in ECs metabolism, mitochondria are crucial for ECs signaling and function and modulate, among other processes, the

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generation of mitochondrial ROS (mtROS).[9,12,13] In particular, mitochondria-dependent redox balance has been recognized important in postischemic neovascularization,[14] wherein mtROS are critical to promote VEGF (vascular endothelial growth factor)-mediated activation of PI3K (phosphatidylinositol 3-kinase)/Akt signaling in ECs.[15–17] Emerging evidence also suggests that balance in mitochondrial dynamics is relevant to ECs function, and its alterations are observed in the endothelium of patients with cardiovascular risk factors.[4,18]

We have previously shown that a family of related mitoAKAPs (mitochondrial PKA anchor proteins) is involved in the amplification within the cell of signal cues to mitochondria.[19–22] MitoAKAPs are generated by alternative splicing of the *Akap1* gene and share a mitochondrial localization domain at NH_2 terminus.[21,23] In multiple cellular systems and tissues, the best-characterized *Akap1* product, AKAP121, regulates mitochondrial structure and function and promotes cell survival under stressful conditions.[20,21] Previous studies from our laboratory and others have shown that AKAP121 undergoes rapid proteolytic degradation on hypoxia.[20–22] Hypoxia-induced degradation of mitoAKAPs impairs mitochondrial function in heart and isolated cardiomyocytes,[20,24] ultimately increasing infarct size and reducing survival after coronary artery ligation in mice.[19,20] More recently, we have demonstrated that *Akap1* controls mTOR (mechanistic target of rapamycin) in cancer cells, modulating cell growth.[25] mTOR complex is a downstream effector of PI3K/Akt and, as discussed before, PI3K/Akt/mTOR signaling pathway plays a key role in angiogenesis regulating ECs function.[26] Other AKAPs have been recognized important in vascular development[27,28] and other cardiovascular diseases.[29–31] However, whether *Akap1* might play a role in ECs biology is currently unknown. In the present study, we hypothesized that mitoAKAPs encoded by the *Akap1* gene might be crucial regulators of endothelial cell behavior.

Methods

The data that support the findings of this study are available from the corresponding authors on reasonable request.

A detailed description of experimental procedures (blood pressure measurements, vascular reactivity studies, ex vivo vascular transfection and superoxide staining, mouse model of hindlimb ischemia and perfusion imaging, assessment of limb function and ischemic damage, serum and urine biochemistry, histological evaluation, isolation of primary mouse endothelial cells, in vitro hypoxia and cells transfection, endothelial cell migration assay, capillary-like network formation on matrigel, endothelial cell proliferation, protein extraction and immunoblot analysis, measurement of NO, mitochondrial membrane potential evaluation, mitochondrial ROS production, annexin V and propidium iodide staining, and electron microscopy) is available in the online-only Data Supplement.

Experimental Animals

All experiments involving animals in this study were conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (8th edition, update 2011) and were approved by the animal welfare regulation of the University of Naples "Federico II", Italy. *Akap*1 knockout mice (*Akap*1$^{-/-}$, C57BL/6 background) were kindly donated by G.S. McKnight and have been previously described.[20,32] Wild-type (wt) and *Akap*1$^{-/-}$ mice of either sex (8–9-week-old) were included in the study and maintained under identical conditions of temperature (21±1°C), humidity (60±5%), and light/dark cycle and had free access to normal mouse chow.

Statistical Analysis

All data presented are expressed as mean±SEM and are representative of ≥3 independent experiments. Comparisons between 2 groups were performed using nonparametric test (Mann–Whitney test). For experiments including ≥3 experimental groups, comparisons were made by 1-way ANOVA or 2-way ANOVA, and *P* values shown indicate the effect of genotype response. Correction for multiple comparisons was made using the Student–Newman–Keuls method. A minimum value of $P<0.05$ was considered statistically significant. All statistical analyses were conducted with Prism statistical software.

Results

Impaired Vascularization and Functional Recovery After Hindlimb Ischemia in Akap1$^{-/-}$ Mice

Previous studies have linked mitochondrial dysfunction to impaired angiogenic response.[16,17] To investigate the role of *Akap1* in neovascularization and endothelial function in vivo, hindlimb ischemia was surgically induced in both wt and *Akap1*$^{-/-}$ mice as previously described.[33,34] Consistent with our previous results in ischemic hearts and hypoxic cardiomyocytes,[20] AKAP121 levels were significantly reduced in wt ischemic limbs (IL) 1 day after femoral artery ligation and remained lower compared with nonischemic limbs up to 28 days after surgery (Figure 1A).

No basal differences in limb perfusion were observed between wt and *Akap1*$^{-/-}$ mice (Figure 1B, pre), and hindlimb perfusion similarly dropped in both genotypes after surgery (Figure 1B, post). However, blood flow recovery in the ischemic hindlimbs of *Akap1*$^{-/-}$ mice was significantly impaired compared with wt at 7, 14, and 28 days after surgery (Figure 1B). Accordingly, ambulatory impairment and ischemic tissue damage of the IL were significantly higher in *Akap1*$^{-/-}$ mice compared with wt (Figure 1C and 1D). Twenty-eight days after surgery, capillary density in IL of *Akap1*$^{-/-}$ mice was significantly reduced compared with wt (Figure 1E). Interestingly, *Akap1*$^{-/-}$ deletion did not affect the rate of interstitial fibrosis after femoral artery ligation that was similarly increased in both wt and *Akap1*$^{-/-}$ (Figure S1 in the online-only Data Supplement). Collectively, these data indicate that *Akap1* is required for postischemic neovascularization.

Because Akt phosphorylation is critical for ECs survival and proliferation under hypoxic conditions,[35] we next evaluated Akt expression and phosphorylation in IL. Interestingly, a blunted response in Akt phosphorylation was observed in IL muscles from *Akap1*$^{-/-}$ mice compared with wt (Figure 1F), suggesting that *Akap1* may be required for Akt phosphorylation in hindlimb ischemia.

Akap1$^{-/-}$ Endothelial Cells Exhibit Alterations in Mitochondrial Structure and Function

Impaired postischemic vascularization and reduced capillary density in *Akap1*$^{-/-}$ mice suggested that *Akap1* deletion might affect ECs behavior. CD31-positive (CD31^{+}) ECs were isolated from aorta of both wt and *Akap1*$^{-/-}$ mice (Figure S2A). In wt ECs, 3 hours of hypoxia significantly induced AKAP121 downregulation (Figure S2B). To determine whether absence of *Akap1* might play a role in mitochondrial structure and function of endothelial cells, we analyzed mitochondrial morphology by electron microscopy in primary ECs from *Akap1*$^{-/-}$ mice or their wt littermates either in normoxia or after 4-hour

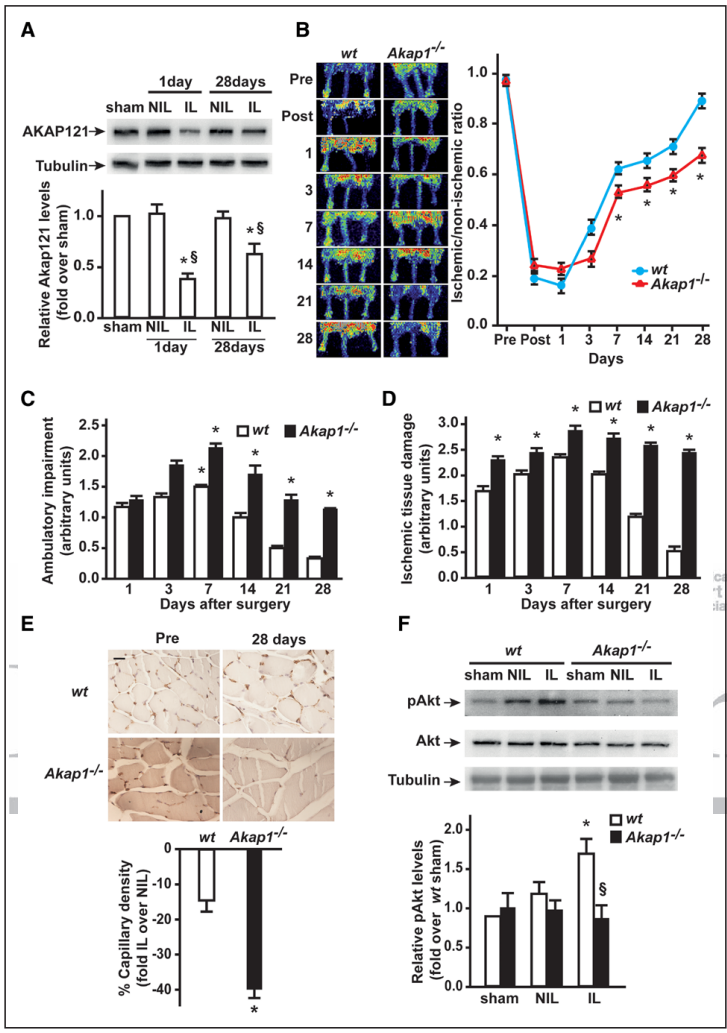

Figure 1. *Akap1* gene deletion impairs hindlimb blood flow and functional recovery after femoral artery ligation. **A**, Representative immunoblot (**top**) and densitometric analysis (**bottom**) of 5 independent experiments to evaluate AKAP121 protein levels in wild-type (wt) skeletal muscle 1 d or 28 d after sham procedure or femoral artery ligation, in ischemic limb (IL) and contralateral nonischemic limb (NIL; **P*<0.05 vs sham; n=5 adductor muscle/group). Tubulin protein levels did not significantly change among the samples. **B**, Representative laser Doppler images (**left**) and graphic curves of blood flow quantification (**right**) in wt and *Akap1* knockout (*Akap1*$^{-/-}$) mice before (Pre), immediately after (Post), and 1, 3, 7, 14, 21, or 28 d after femoral artery ligation (**P*<0.05 vs wt IL; n=8 animals/group). **C**, Cumulative data of ambulatory impairment of wt and *Akap1*$^{-/-}$ mice 1, 3, 7, 14, 21, and 28 days after femoral artery ligation (**P*<0.05 vs wt IL; n=8 animals/group). **D**, Cumulative data of ischemic tissue damage of wt and *Akap1*$^{-/-}$ mice 1, 3, 7, 14, 21, and 28 d after femoral artery ligation (**P*<0.05 vs wt IL; n=8 animals/group). **E**, Representative lectin staining (**top**) and bar graphs (**bottom**) of cumulative data of multiple independent experiments analyzing capillary density in wt and *Akap1*$^{-/-}$ muscles before (Pre) and 28 d after femoral artery ligation. Capillaries appear brown (**P*<0.05 vs wt ischemic limb; n=5 adductor muscle/group; bar=20 μm). **F**, Representative immunoblots (**top**) and densitometric analysis (**bottom**) of 4 independent experiments to evaluate Akt phosphorylation levels (Ser-473) in adductor muscles of wt and *Akap1*$^{-/-}$ mice after sham procedure or 1 d of femoral artery ligation. Relative pAkt levels (fold over wt sham) are reported. Tubulin and Akt protein levels did not significantly change among the samples; pAkt levels were normalized by tubulin levels (**P*<0.05 vs wt sham; §*P*<0.05 vs wt ischemic limb; n=6 adductor muscle/group).

hypoxia. In normoxic wt ECs, mitochondria displayed normal size and shape (Figure 2A, top left). In contrast, mitochondria in normoxic *Akap1*$^{-/-}$ ECs were characterized by significant alterations in the architecture of mitochondrial cristae and matrix dilution (Figure 2A, bottom left). After 4-hour hypoxia, mitochondrial structure was significantly altered in wt ECs,

and these abnormalities were significantly more pronounced in *Akap1*$^{-/-}$ cells (Figure 2A, right). The percentage of number of mitophagosomes was significantly increased in *Akap1*$^{-/-}$ ECs (Figure 2B). Moreover, compared with wt cells, mitochondrial membrane potential measured by tetramethylrhodamine ethyl ester fluorescence was decreased in *Akap1*$^{-/-}$ ECs under normoxia and further decreased in hypoxia (Figure 2C). Consistent with these results, mtROS production was significantly increased in *Akap1*$^{-/-}$ ECs compared with wt in normoxia, and this difference was further increased under hypoxic conditions (Figure 2D). These data suggest that *Akap1* is crucial for mitochondrial structure and function in ECs and that its deletion directly promotes mitochondrial damage and contributes to the exacerbation of hypoxia-induced mitochondrial alterations.

Reduced Akt Activation and Enhanced Apoptosis in Akap1$^{-/-}$ Endothelial Cells

Hypoxia- or VEGF-induced phosphorylation of Akt controls vascular homeostasis and ECs survival.[36] As expected, 3/6 hours of hypoxia or short-term VEGF stimulation induced a significant increase in Akt phosphorylation in wt ECs, whereas this response was significantly inhibited in *Akap1*$^{-/-}$ cells (Figure 3A and 3B). Because eNOS phosphorylation by Akt promotes its activation, increasing NO production,[7] we next tested NO levels in the cell culture media of *Akap1*$^{-/-}$ and wt ECs after acetylcholine stimulation. Although in wt ECs, NO levels significantly increased after acetylcholine stimulation, in *Akap1*$^{-/-}$ cells, this response was significantly blunted (Figure 3C). Taken together, these results demonstrate that cytoprotective Akt-mediated signaling is significantly reduced after *Akap1* genetic deletion. Consistent with these results, hypoxia-induced cell death was significantly higher in *Akap1*$^{-/-}$ ECs compared with wt, as demonstrated by Annexin V/propidium iodide staining (Figure 3D) and caspase 3 activation (Figure 3E).

ECs Alterations Induced by Akap1 Deletion Are Reversed by Akt Constitutive Activation

We next investigated the role of *Akap1* in ECs proliferation, network formation, and migration. Compared with wt, the proliferation rate of *Akap1*$^{-/-}$ ECs was significantly lower (Figure 4A, gray squares and black squares, respectively).

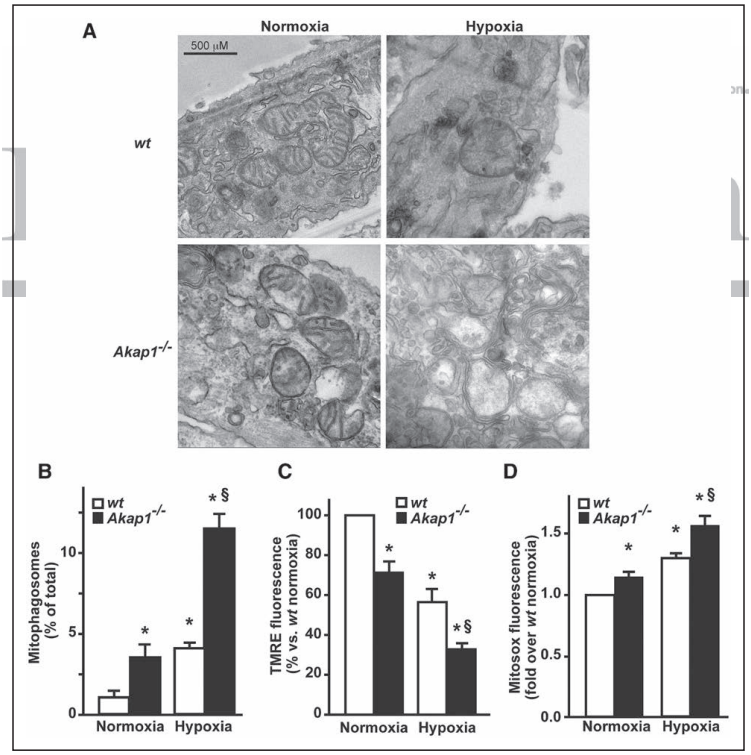

Figure 2. *Akap1* genetic deletion promotes mitochondrial morphological and functional alterations in primary murine aortic endothelial cells. **A**, Representative electron microscopy images of mitochondria in endothelial cells (ECs) isolated from wild-type (wt) or *Akap1* knockout (*Akap1*$^{-/-}$) mice and incubated in normoxic or hypoxic conditions (bar=500 μm; n=3 independent experiments). Bar graphs showing cumulative data of multiple independent experiments to quantify percentage of mitophagosomes (**B**), mitochondrial membrane potential by tetramethylrhodamine ethyl ester (TMRE) fluorescence (**C**), and mitochondrial reactive oxygen species generation by Mitosox staining (**D**) in wt or *Akap1*$^{-/-}$ ECs under normoxic or hypoxic conditions (each experiment was performed in triplicate; for all panels, **P*<0.05 vs wt normoxia; §*P*<0.05 vs wt hypoxia; n=3 independent experiments).

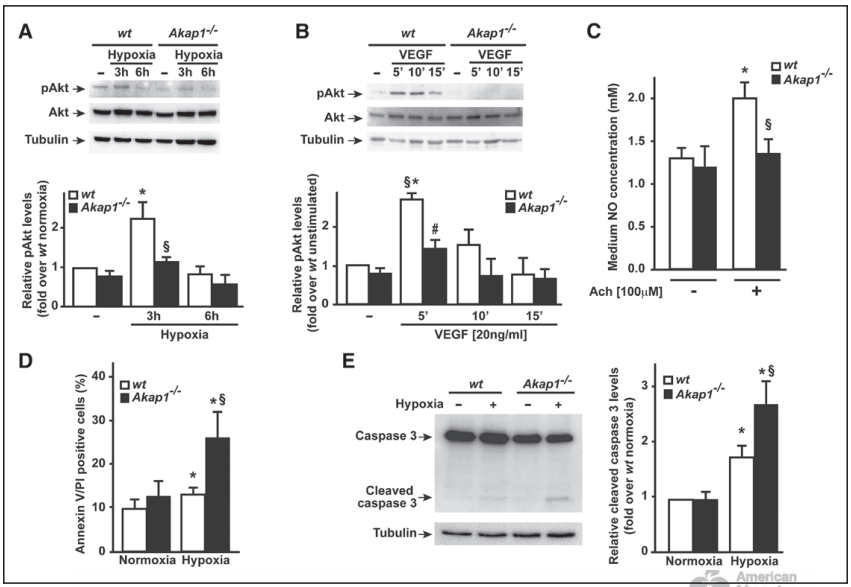

Figure 3. Reduced activation of Akt and enhanced apoptosis in *Akap1* knockout (*Akap1*$^{-/-}$) endothelial cells (ECs). **A**, Representative immunoblots (**top**) and densitometric analysis (**bottom**) of 4 independent experiments to evaluate total Akt levels and Akt phosphorylation levels at Ser-473 in wild-type (wt) and *Akap1*$^{-/-}$ ECs in normoxic or hypoxic conditions for 3 or 6 h. Relative pAkt levels (fold over wt normoxia) are reported. Tubulin and Akt protein levels did not significantly change among the samples; pAkt levels were normalized by tubulin levels (*P<0.05 vs wt normoxia, §P<0.05 vs wt 3-h hypoxia). **B**, Representative immunoblots (**top**) and densitometric analysis (**bottom**) of 4 independent experiments to evaluate total Akt levels and Akt phosphorylation levels at Ser-473 in wt and *Akap1*$^{-/-}$ ECs after VEGF (vascular endothelial growth factor) stimulation (20 ng/mL) for 5, 10, or 15 min. Relative pAkt levels (fold over unstimulated wt cells) are reported. Tubulin and Akt protein levels did not significantly change among the samples; pAkt levels were normalized by tubulin levels (*P<0.05 vs wt (–); #P<0.05 vs *Akap1*$^{-/-}$ (–), §P<0.05 vs *Akap1*$^{-/-}$ VEGF stimulated). **C**, Nitrite levels measured in supernatants of wt and *Akap1*$^{-/-}$ ECs after acetylcholine stimulation (Ach, 100 μmol/L; n=3 independent experiments; *P<0.05 vs wt (–); §P<0.05 vs wt Ach-stimulated). **D**, Cumulative data of 3 independent experiments measuring Annexin V and propidium iodide (PI) staining of ECs in normoxic or hypoxic conditions for 3 h (*P<0.05 vs wt normoxia; §P<0.05 vs wt hypoxia) **E**, Representative immunoblots (**left**) and densitometric analysis (**right**) of 4 independent experiments to evaluate caspase 3 activation in wt and *Akap1*$^{-/-}$ ECs in normoxic or hypoxic conditions for 3 h. Tubulin protein levels did not significantly change among the samples (*P<0.05 vs wt normoxia; §P<0.05 vs wt hypoxia).

This difference was statistically significant 24 hours after starting the assay (mean number of cells±SEM=15 733±1059 wt versus 9433±328 *Akap1*$^{-/-}$) and persisted after 48 hours (35 200±2294 wt versus 13 833±727 *Akap1*$^{-/-}$) and 72 hours (70 400±3553 wt versus 20 933±612 *Akap1*$^{-/-}$; for all, P≤0.05 wt versus all). Interestingly, pretreatment with a selective Akt inhibitor (Akt-i) significantly reduced the proliferation rate of wt ECs (Figure 4A, gray dots), whereas it did not exert any additional effects in *Akap1*$^{-/-}$ ECs (Figure 4A, black dots). Conversely, transient transfection of *Akap1*$^{-/-}$ ECs with a plasmid encoding for a constitutively active, myristoylated Akt (Myr-Akt),[37] partially restored the proliferation rate in these cells (Figure 4A, white dots). As expected, Myr-Akt construct increased Akt phosphorylation in *Akap1*$^{-/-}$ ECs (Figure S3).

VEGF-induced capillary-like network formation was also significantly impaired in *Akap1*$^{-/-}$ ECs compared with wt (Figure 4B). Pretreatment with Akt-i significantly reduced network formation in wt ECs, whereas Myr-Akt transfection restored *Akap1*$^{-/-}$ tubes formation (Figure 4B). Cell migration of *Akap1*$^{-/-}$ ECs was also significantly compromised compared with wt (Figure 4C). Pretreatment of wt ECs with Akt-i significantly reduced cell motility of wt ECs, whereas Myr-Akt transfection significantly increased mean cover area in *Akap1*$^{-/-}$ cells (Figure 4C). Taken together, these data demonstrate that *Akap1* deletion impairs multiple in vitro functions of endothelial cells, and these abnormalities are mediated, at least in part, by reduced Akt signaling.

Akap1 Deletion Induces Endothelial Dysfunction in Murine Resistance Arteries and Regulates Arterial Blood Pressure

Given the robust defects in structure and function observed in isolated *Akap1*$^{-/-}$ ECs, we sought to determine whether absence of *Akap1* might directly affect vascular function. To evaluate the presence of vascular alterations and to distinguish between endothelium-dependent and -independent vasodilatatory effects, preconstricted vascular segments of aorta, mesenteric arteries, and femoral arteries from *Akap1*$^{-/-}$ or wt mice were treated with either acetylcholine or nitroglycerin (Figure 5A and 5B; Figure S4A through S4D). Interestingly, compared with wt, *Akap1*$^{-/-}$ mice were characterized by a marked reduction in acetylcholine-induced vasorelaxation in mesenteric arteries (Figure 5A), with no significant differences in nitroglycerine-treated vessels (Figure 5B) or in aortic

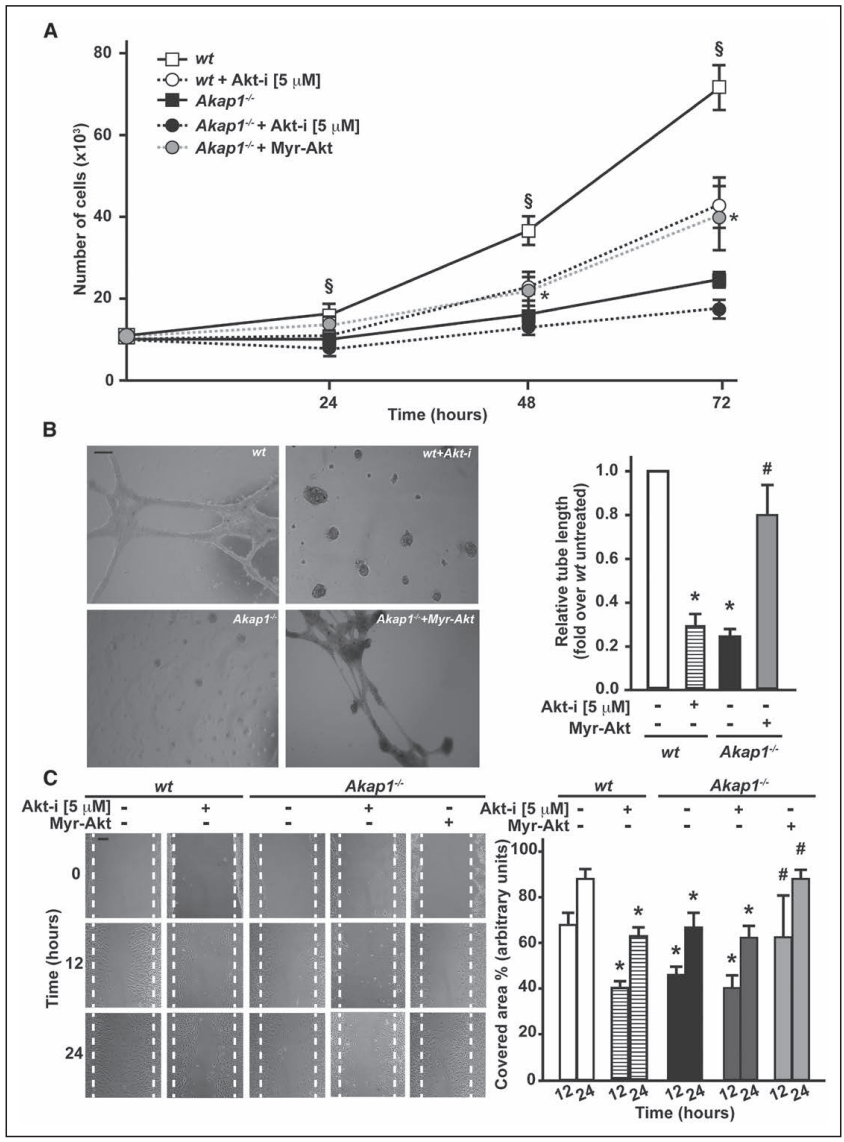

Figure 4. Constitutive Akt activation ameliorates proliferation, migration, and capillary network formation in *Akap1* knockout (*Akap1*$^{-/-}$) endothelial cells (ECs). **A**, Cumulative data representing 5 independent experiments to measure proliferation rates of wild-type (wt) and *Akap1*$^{-/-}$ ECs 24, 48, and 72 h after plating (for all groups, 10^3 cells/well) in the presence or absence of an Akt inhibitor (Akt-i, 5 μmol/L) or 24 h after transient transfection with Myr-Akt. Each experiment was performed in triplicate (§P<0.05 wt vs all; *P<0.05 *Akap1*$^{-/-}$ vs *Akap1*$^{-/-}$ + Myr-Akt). **B**, Representative capillary-like network formation (**left**) and bar graphs (**right**) showing quantification of relative tube length from 5 independent experiments performed in wt and *Akap1*$^{-/-}$ ECs in presence or absence of Akt-i (5 μmol/L) or transfected with myristoylated Akt (Myr-Akt) construct (n=6 independent experiments; *P<0.05 vs wt; #P<0.05 vs. *Akap1*$^{-/-}$; bar=50 μm). **C**, Representative images (**left**) and bar graphs quantification (**right**) of wt and *Akap1*$^{-/-}$ EC migration in the presence or absence of Akt-i (5 μmol/L) or after transfection with Myr-Akt, at time 0, 12, and 24 h after wound injury (n=6 independent experiments; *P<0.05 vs wt; #P<0.05 vs. *Akap1*$^{-/-}$; bar=20 μm).

rings and femoral arteries response (Figure S4A through S4D), suggesting a selective impairment in endothelium-dependent relaxation. Phenylephrine- or norepinephrine-induced vasoconstriction was similar between the 2 groups in all vascular segments (Figure 5C; Figure S4E through S4H; data not shown).

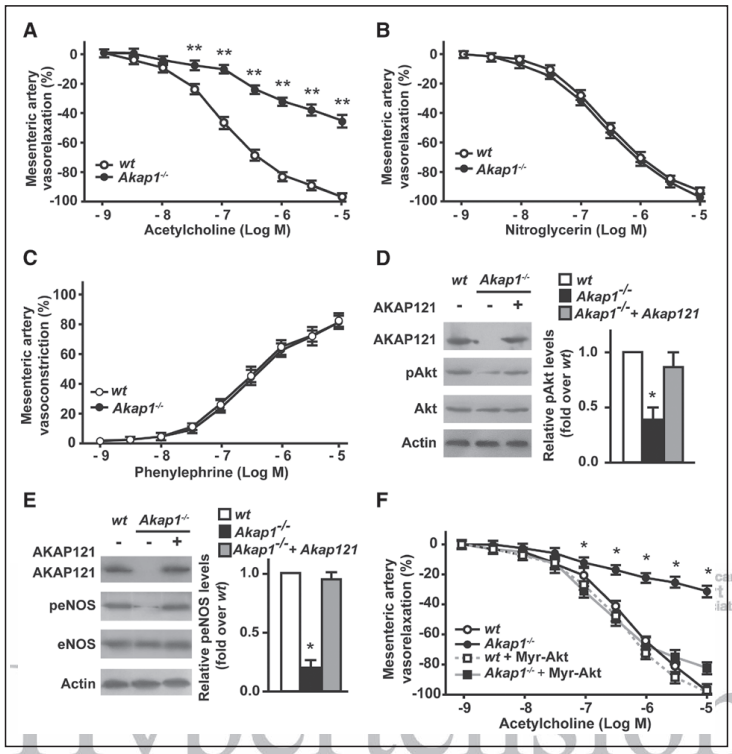

Figure 5. *Akap1* genetic deletion impairs Akt and endothelial NO synthase (eNOS) phosphorylation and reduces acetylcholine-induced vasorelaxation in mesenteric arteries. Dose–response curves of phenylephrine-precontracted wild-type (wt) and *Akap1* knockout (*Akap1*$^{-/-}$) mesenteric arteries (n=9 mice/group) to acetylcholine (**A**) and nitroglycerin (**B**; **P<0.01 vs wt). **C**, Dose–response curves of mesenteric artery vasoconstriction of wt and *Akap1*$^{-/-}$ mesenteric arteries treated with phenylephrine (p=ns; n=9 mice/group). **D**, Representative immunoblots (**left**) of AKAP121, pAkt, Akt, and actin proteins in wt and *Akap1*$^{-/-}$ mesenteric arteries before and after transfection with constructs encoding for AKAP121. Densitometric analysis of relative pAkt levels (fold over wt) are reported on the **right**. Actin and Akt protein levels did not significantly change among the samples; pAkt levels were normalized by actin levels (n=3 independent experiments; *P<0.01 vs all). **E**, Representative immunoblots (**left**) of AKAP121, phospho-eNOS (peNOS), eNOS, and actin proteins in wt and *Akap1*$^{-/-}$ mesenteric arteries before and after transfection with constructs encoding for AKAP121. Densitometric analysis of relative peNOS protein levels (fold over wt) are reported on the **right**. Actin and eNOS protein levels did not significantly change among the samples (n=3 independent experiments; *P<0.01 vs. all). **F**, Acetylcholine-induced vasorelaxation of mesenteric arteries from wt and *Akap1*$^{-/-}$ mice transiently transfected with myristoylated Akt (Myr-Akt) construct or vehicle (*P<0.05 vs. all; n=9 mice/group).

Endothelium-derived NO synthesis is critical in the regulation of vasorelaxation and blood pressure, and eNOS phosphorylation by Akt is a major event for its activation,[7] partially regulated by ROS in a dose- and time-dependent manner.[38] Absence of *Akap1* increased ROS production (Figure S5) and significantly reduced the phosphorylation of both Akt and eNOS in mesenteric arteries (Figure 5D and 5E). Importantly, phosphorylation of both enzymes was completely restored after transient transfection of *Akap1*$^{-/-}$ vessels with DNA constructs encoding for AKAP121,[21] confirming the specific role of *Akap1* gene deletion in Akt and eNOS alterations (Figure 5D and 5E). The defect in acetylcholine-induced vasorelaxation of mesenteric arteries from *Akap1*$^{-/-}$ mice was restored after transient transfection with Myr-Akt (Figure 5F). Collectively, these data suggest a direct effect of *Akap1* in the regulation of endothelial-dependent vascular function through modulation of Akt–eNOS axis.

Finally, to test whether the endothelial dysfunction observed in *Akap1*$^{-/-}$ mice might affect systemic blood pressure regulation in vivo, we monitored arterial blood pressure in *Akap1*$^{-/-}$ and wt mice. Using both tail-cuff recording and direct intra-arterial measurements, we observed that *Akap1*$^{-/-}$ mice displayed a mild, albeit significant, increase in blood pressure levels compared with their wt littermates (Figure 6; Table S1 in the online-only Data Supplement). Interestingly, *Akap1* heterozygous mice (*Akap*$^{+/-}$) also displayed an intermediate phenotype (Table S1). To rule out the possible contribution of renal dysfunction in the development of vascular phenotype in *Akap1*$^{-/-}$ mice, we performed a biochemical analysis of the most common serum and urine markers of renal function in these mice. As shown in Table S2, no significant differences in kidney function were found between *Akap1*$^{-/-}$ mice and their wt littermates. Consistent with the functional data, no alterations were found in the renal

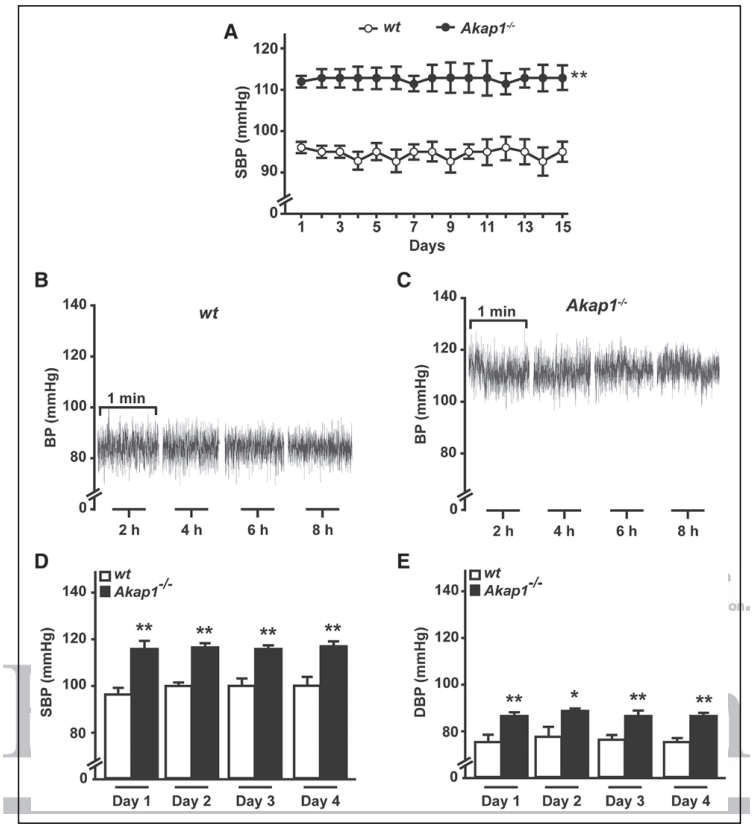

Figure 6. Arterial blood pressure alterations in *Akap1*$^{-/-}$ mice. **A**, Arterial systolic blood pressure (SBP) of wild-type (wt) and *Akap1* knockout (*Akap1*$^{-/-}$) mice measured by tail-cuff plethysmography (**P<0.01 vs wt; n=9 mice/group). Representative intrafemoral blood pressure (BP) traces (1 minute) measured in wt (**B**) or *Akap1*$^{-/-}$ (**C**) mice at 2-, 4-, 6-, and 8-h time points. Bar graphs reporting cumulative analysis of daily measurements of invasive SBP (**D**) and diastolic blood pressure (DBP, **E**) in wt and *Akap1*$^{-/-}$ mice (**P<0.01 and *P<0.05 vs. wt; n=4 mice/group/d).

architecture of *Akap1*$^{-/-}$ mice compared with wt littermates, with evidence of normal nephrons structure in both genotypes (Figure S6) and absence of differences in the ratio between kidney weight and body weight (Table S2). Taken together, these results suggest that the observed effects on blood pressure control could not be attributed to major effects on renal function in *Akap1*$^{-/-}$ mice.

Discussion

Orchestrators of mitochondrial function in ECs are still poorly understood. In the present study, we demonstrate that mitochondria-targeted AKAP proteins encoded by the *Akap1* gene play a fundamental role in modulating ECs behavior and vascular function, at least partially through the modulation of Akt activity. Our data suggest that absence of *Akap1* profoundly affects mitochondrial structure and function in ECs, resulting in mitochondrial degradation through mitophagy, reduced mitochondrial membrane potential, and increased mtROS production. Mitochondrial alterations observed in *Akap1*$^{-/-}$ ECs significantly impact on multiple endothelial functions in vitro. Importantly, *Akap1*$^{-/-}$ mice exhibit impaired NO-dependent vascular relaxation, reduced postischemic neovascularization, and increased blood pressure levels. As far as we are aware, this study provides the first evidence that alterations in mitochondrial cAMP-dependent signaling affects ECs behavior and vascular reactivity in vitro and in vivo, presenting a new conceptual framework to understand and treat mitochondrial dysfunction in vascular diseases.

Because ECs mostly rely on glycolysis to cope with their energy demands, the canonical role of mitochondria as energy power has been previously challenged in these cells. However, accumulating evidence identify mitochondria as important signaling organelles in endothelium,[39,40] and it has been increasingly recognized that mitochondrial alterations participate in endothelial dysfunction.[4] Mitochondrial signaling network is complex and strictly regulated. Others and we have previously shown the essential role of mitochondrial cAMP signaling, orchestrated by mitoAKAPs, in regulating

mitochondrial function and stress response in different type of cells.[19–22,24,41,42] Although cAMP and its main effector PKA participate in several vascular processes[43–45] and different AKAPs have been involved in vascular function,[46–48] the role of mitoAKAPs in regulating ECs function and vascular properties is still unknown.

In our study, mice with *Akap1* genetic deletion exhibit delayed skeletal muscle revascularization after ischemic insult, vascular dysfunction with impaired NO-dependent relaxation, and alteration in blood pressure regulation. These findings suggest that absence of *Akap1* significantly impacts on endothelial function at multiple levels, affecting vascular tone, blood pressure levels, and postischemic neovascularization. Importantly, because blood pressure level analysis using tail-cuff method can exhibit variability among different measurements depending (mostly) on the mouse body temperature at the moment of recording, we also confirmed our data using direct invasive intra-arterial measurements. Interestingly, renal structure and function was normal in *Akap1*$^{-/-}$ mice, suggesting that the observed effects on blood pressure and vasculature are related to a primary dysfunction of vascular ECs. Despite the increase in arterial blood pressure levels in *Akap1*$^{-/-}$ mice compared with their wt littermates, no differences were detected among the groups in cardiac fibrosis[20] and in both systolic[20] and diastolic left ventricular function under basal conditions (Table S3). The normal phenotype of adult *Akap1*$^{-/-}$ mice can be explained by the fact that other mitochondria-targeted AKAP-like proteins, like Rab32, might compensate for the absence of *Akap1*.[49,50]

Our data demonstrate that the absence of *Akap1* leads to impaired endothelial function and reduced eNOS activity. Given the global deletion of *Akap1*, the observed reduction in eNOS phosphorylation has to occur in endothelial cells in every single organ, including heart and kidney. The fact that, despite the reduced levels of eNOS, *Akap1*$^{-/-}$ mice did not show any baseline cardiac or renal alterations is consistent with the literature published on eNOS knockout mice (*eNOS*$^{-/-}$). Global deletion of eNOS does not affect cardiac function, neither systolic nor diastolic function,[51,52] or renal structure and function.[53,54] Therefore, the available evidence of *eNOS*$^{-/-}$ mice pointed to the direction that the complete absence of eNOS, despite the increase in blood pressure, is not sufficient to cause any baseline cardiac or renal phenotype. Hence, it is not surprising that no obvious differences in organ structure and function were detected in *Akap1*$^{-/-}$ mice, in which a residual activity of eNOS is still present, at least during a short-term observation period. However, it is not possible to exclude that later during life these animals might develop pathophysiological changes inducing alterations in cardiac (in particular diastolic) and renal function. Further studies will be needed to uncover additional possible long-term pathological phenotypes in *Akap1*$^{-/-}$ mice.

Moving forward, we sought to determine the molecular mechanisms involved in the *Akap1*-dependent regulation of endothelial function. Interestingly, a recent study reported that *Akap1* participates in VEGF signaling through PKA-dependent phosphorylation of VEGF receptor II (VEGFRII).[55] In our cellular system, VEGFR2 levels in *Akap1*$^{-/-}$ ECs were similar to wt cells, and VEGF-stimulated autophosphorylation of the cytosolic domains of VEGFR2 were also not significantly different between *Akap1*$^{-/-}$ and wt ECs (data not shown). Despite this, VEGF-induced Akt phosphorylation was significantly reduced in *Akap1*$^{-/-}$ ECs compared with wt, suggesting that downstream effectors of VEGF pathway are altered in *Akap1*$^{-/-}$ ECs. Although VEGF represents one of the most potent stimuli for ECs, we observed reduced Akt activation in *Akap1*$^{-/-}$ ECs also in response to hypoxia, suggesting that different stimuli, and perhaps other growth factors, might rely on mitoAKAPs as intermediate signaling molecules in ECs. *Akap1* genetic deletion also significantly reduced phosphorylation of Akt and eNOS in mesenteric arteries, and a defect in hypoxia-induced phosphorylation of Akt was also observed in *Akap1*$^{-/-}$ skeletal muscles after femoral artery ligation. The PI3K/Akt/mTOR signaling pathway is known to be important for vascular function and in particular for ECs survival. mTOR was recently found as a downstream target of *Akap1* in cancer cells[25]; however, the precise molecular mechanisms linking mitoAKAPs to mTOR-upstream activators, such as Akt, remain still elusive and deserve future investigations. In the present study, the myristoylated, constitutively active form of Akt (Myr-Akt) restored vascular relaxation in *Akap1*$^{-/-}$ mesenteric arteries, suggesting Akt as a mediator of vascular effects in *Akap1*$^{-/-}$ mice. Consistent to what observed in isolated vessels, Myr-Akt also improved proliferation, migration, and tube formation in *Akap1*$^{-/-}$ ECs, suggesting that the amelioration of the vascular phenotype observed with Myr-Akt in the whole vessel, is attributable, at least in part, to the beneficial effect observed in ECs. Collectively, the evidence that in absence of *Akap1*, both ex vivo vascular relaxation and in vitro endothelial function improve after transfection with a constitutively active form of Akt, confirms the involvement of Akt as a part of *Akap1* downstream signaling complex in response to stress in ECs. We propose that *Akap1* might regulate eNOS activity through its effects on Akt. Hence, *Akap1* deletion might result in reduced Akt phosphorylation and, in turn, eNOS activation. However, because a direct regulation of eNOS by PKA has been also previously reported,[56] it is not possible to exclude that reduced eNOS activation in *Akap1*$^{-/-}$ ECs might rely on a direct PKA-dependent mechanism.

As discussed, endothelial dysfunction concurs in the development of most cardiovascular diseases. Although several lines of evidence show that mitochondria are important for ECs function, only few translational studies have specifically addressed the impact of mitochondrial alterations on vascular function. This observation prompts questions on the potential use of mitochondria-directed therapeutic interventions for treatment of cardiovascular disease. To date, scavengers for mtROS and activators of AMPK (AMP-activated protein kinase)/PGC-1α (peroxisome proliferator-activated receptor γ coactivator 1-α) have shown promising results also in endothelium.[57–59] Because mitoAKAPs have been shown to be critical for cellular homeostasis and proper response to stress in several cell types, including cardiomyocytes, pharmacological approaches that enhance *Akap1* mitochondrial targeting may provide beneficial effects in cardiovascular diseases through a broad spectrum of cell-specific effects.

Perspectives

EC dysfunction is a hallmark of several cardiovascular diseases, including arterial hypertension. The precise mechanisms

underlying these abnormalities are still not completely understood. Mitochondria are important regulators of crucial cellular functions, and mitochondrial dysfunction may affect ECs behavior. Here, we show the effects of the *Akap1* gene, encoding for a family of mitoAKAPs, on ECs behavior in vitro and in vivo. Compared with their wt littermates, *Akap1*$^{-/-}$ exhibit impaired vascular relaxation, reduced ischemia-induced neovascularization, and a mild increase in blood pressure levels. *Akap1* deletion induces a significant enhancement of hypoxia-induced mitochondrial dysfunction and apoptosis. In addition, capillary-like network formation, migration, and proliferation are remarkably impaired in *Akap1*$^{-/-}$ ECs. Here, we recognize a new role for mitoAKAPs in ECs biology, suggesting an association between mitochondrial alterations and endothelial dysfunction and pointing at *Akap1* as a novel therapeutic target for treating vascular diseases.

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Disclosures

None.

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Novelty and Significance

What Is New?

- Genetic deletion of *Akap1* impairs multiple endothelial cell functions causing vascular alterations and modulating blood pressure.
- Endothelial alterations induced by *Akap1* deletion can be restored by a constitutively active form of Akt.

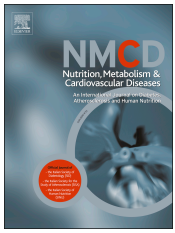
What Is Relevant?

- Vascular alterations are common in many cardiovascular diseases.
- Mitochondrial alterations have been associated with endothelial dysfunction.
- Our findings indicate a previously unrecognized role for mitoAKAPs (mitochondrial A kinase anchoring proteins) in modulating multiple functions of endothelial cells.

Summary

MitoAKAPs govern endothelial cells behavior and might represent novel therapeutic targets for cardiovascular disease characterized by endothelial dysfunction.

Journal Pre-proof



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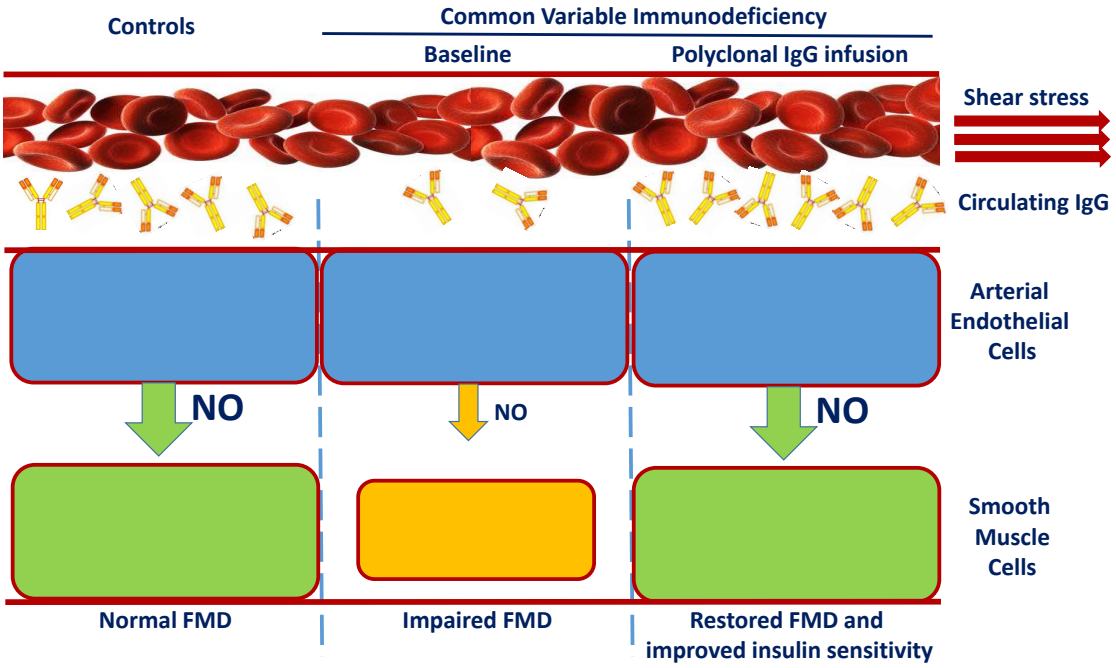
Controls
Common Variable Immunodeficiency
Baseline
Polyclonal IgG infusion
Shear stress
Circulating IgG
Arterial Endothelial Cells
NO
NO
NO
Smooth Muscle Cells
Normal FMD
Impaired FMD
Restored FMD and improved insulin sensitivity

Immunoglobulins G Modulate Endothelial Function and affect Insulin Sensitivity in Humans

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Conflict of interest

The authors have no conflict of interest to disclose.

Key words.
Atherosclerosis, Endothelial function, Flow Mediated Dilation, Immunoglobulins, Insulin resistance

Abstract

Background and Aims. Data from animals suggest that immunoglobulins G (IgG) play a mechanistic role in atherosclerosis and diabetes through endothelial dysfunction and insulin resistance. Patients with common variable immunodeficiency (CVID), who have low circulating levels of IgG and are treated with intravenous polyclonal IgG (IVIgG), may provide an ideal model to clarify whether circulating IgG modulate endothelial function and affect insulin sensitivity in humans.

Methods and Results. We studied 24 patients with CVID and 17 matched healthy controls (HC). Endothelial function was evaluated as flow mediated dilation (FMD) of the brachial artery at baseline and 1, 7, 14, and 21 days after IVIgG infusion in the CVID patients. We measured also plasma glucose, insulin, and calculated the HOMA-IR index. We also investigated the role of human IgG on the production of Nitric Oxide (NO) *in vitro* in Human Coronary Artery Endothelial Cells (HCAEC).

Compared to HC, FMD of CVID patients was significantly impaired at baseline (9.4±0.9 and 7.6±0.6% respectively, $p<0.05$) but rose above normal levels 1 and 7 days after IVIgG infusion to return at baseline at 14 and 21 days. Serum insulin concentration and HOMA-IR index dropped by 50% in CVID patients after IVIgG ($p<0.002$ vs baseline). In *vitro* IgG stimulated NO production in HCAEC.

Conclusions. Reduced IgG levels are associated with endothelial dysfunction and IVIgG stimulates endothelial function directly while improving insulin sensitivity. The current findings may suggest an anti-atherogenic role of human IgG.

Introduction

Several receptors for the Fc fragment (FcγR) of circulating immunoglobulins (Ig) have been identified in the last few decades in many different cell types, in both humans and other species [1,2]. Given the importance of endothelial function in the maintenance of vascular health and the prevention of atherosclerosis and cardiovascular diseases (CVD), the observation that two FcγRs, i.e. FcγRI and FcγRIIB, are also expressed in endothelial cells has prompted large interest in the field [3, 5]. The fact that C Reactive protein (CRP) interacts with FcγRs on the endothelial cells makes their role even more relevant. Although the role of circulating CRP in the pathogenesis of CVD has been recently challenged [6,7], its capability of affecting endothelial function, insulin action, and insulin-mediated glucose uptake has been shown in numerous studies [5,8-12]. The impairment of insulin-mediated glucose disposal by CRP has been postulated to be due to an impaired endothelial mediated vasodilation which in turn reduces substrates and insulin delivery to the muscle [9]. On the other hand, FcγRs are constantly and mainly exposed in *vivo* to the stimulation of circulating IgG. Recently, it has been demonstrated in mice that peripheral insulin resistance is due to impaired endocytosis of serum insulin by the endothelial cells, triggered by hyposialylation of circulating IgG resulting in potentiated FcγRIIB stimulation [13]. However, the effects and the role played in humans by circulating IgG on the endothelium, the regulation of peripheral blood flow and insulin sensitivity are unknown.

Patients with common variable immunodeficiency (CVID), who are characterized by a reduction greater than two standard deviations of IgG serum levels due to an impaired or absent antibody production in response to pathogens, might represent an ideal model to clarify the tonic role, if any, played by circulating IgG on vascular function *in vivo* in humans [14, 15]. Although these patients do not show an increased risk for CVD [1,5,14,15], to control their

disease, they need to be treated regularly by administration of intravenous polyclonal IgG (IVIgG) [14, 15]. Such infusions may allow to evaluate the effects of IVIgG and provide an opportunity to clarify the role played by changes in circulating IgG on vascular function, nitric oxide (NO) bioavailability, and insulin sensitivity. Since IgG are constantly flowing in the bloodstream, studying the effects of IVIgG in vivo in humans might support the hypothesis of a tonic role of circulating Ig on endothelial function and NO release.

Therefore, purposes of the current study were to clarify the role that circulating IgG play on endothelial function in *vivo* in humans and whether IgG directly interact with the endothelial cells. Changes in endothelial function, measured as flow mediated dilation (FMD) following the infusion of IVIgG represent the primary endpoint of the study. In the same patients, we measured also the intima-media thickness (IMT) of the carotid arteries. In addition, we evaluated the production of NO in Human Coronary Artery Endothelial Cells (HCAEC) in vitro after incubation with polyclonal IgG. An ancillary purpose of the current study was to evaluate whether circulating IgG affect insulin sensitivity.

Methods

Subjects. We studied 24 patients with CVID (13F/11M, disease duration 18±2 yrs), recruited among the patients referring to the Allergy and Immunodeficiency Unit of the Federico II University hospital. To be recruited in the study, patients had to be diagnosed with CVID and treated with IVIgG for at least 6 months. They had to be free of acute infections or cancer, kidney or liver failure. The CVID patients had been regularly receiving for several years IVIgG infusion (Kiovig©, Baxter AG, Vienna, Austria; Ig Vena©, Kedrion S.p.A., Castelvecchio Pascoli, Barga, Lucca, Italy; Flebogamma DIF©, Instituto Grifols S.A., Parets del Vallès, Barcelona, Spain) every 3 to 5 weeks for the treatment of their disease. Among them, 4 patients suffered from hypertension (2 of them on treatment with ACE inhibitors only, 1 with ACE inhibitor and diuretic, 1 with ARB), 3 from type 2 diabetes (all treated with metformin, 1 of them with DPP4i also, 2 of them had both diseases). No patient was affected by CVD. As a control group, we studied 17 healthy subjects (2F/15M) recruited among hospital staff and visitors (HC group). The study protocol, that conforms to the ethical guidelines of the 1975 Declaration of Helsinki, was approved by the ethics committee of our institution and informed consent was collected from the patients involved in the study.

Study Protocols

Primary endpoint of the study was the change in endothelial function, measured as flow mediated dilation (FMD) following the infusion of IVIgG. To this aim, all the subjects were studied in the morning after an overnight fast. The patients from the CVID group were studied when, for clinical or personal reasons, the interval from the last IVIgG infusion was 5 weeks (baseline visit). On the morning of day 0, endothelial function of the brachial artery, assessed by ultrasonography as flow mediated dilation (FMD), was measured (baseline). Immediately after the FMD measurements and blood collection, the patients received half dose of the IVIgG

infusion necessary for IgG replacement (half of 400 mg/kg body weight in 5-10% solution). 24 hours later, before administering the second half of the dose of the IVIgG, vascular reactivity was again measured (day 1). The dose of IVIgG infusion used in the protocol was the one regularly used by the patients to treat their disease. Vascular reactivity was again measured 1, 2, and 3 weeks after the first IVIgG infusion (Protocol A). To evaluate the acute, early effects of IgG infusion on vascular reactivity, a subgroup of 17 patients were studied again few weeks later, when they had to receive, for therapeutic reasons, a new dose of IVIgG. In this case they were all studied at three-week interval from the previous IVIgG administration. In this occasion, FMD was measured before and 1 hour after the first infusion of polyclonal IgG (Protocol B).

Blood samples were collected from all the patients at baseline and at various time-points thereafter for the measurements of circulating immunoglobulins, high sensitivity CRP (hsCRP), glucose, and insulin. All the laboratory measurements were made in our institution.

Assessment of endothelial function

FMD was assessed according to the guidelines of the International Brachial Artery Reactivity Task Force [16], using a high-resolution ultrasound system (Toshiba Applio XG, Tokyo, Japan) equipped with a 7.5 MHz multifrequency linear-array transducer (range 4-9.2 MHz), as previously described [17]. To obtain the FMD, brachial artery internal diameter (BAD) was measured at baseline and after reactive hyperemia, created by inflating the cuff placed above the antecubital fossa to a pressure of 250 mmHg for 5 min. To improve our reproducibility and reduce measurement bias we used a probe and arm holder. After the patient had rested for at least 10 min, a new baseline BAD was recorded and a sublingual dose of nitroglycerin spray (0.3 mg) was administered to assess non-endothelial-mediated vasodilation (NMD). Brachial artery images were acquired continuously for 4 min and converted into a digital DICOM file and stored

on CD-ROMs for subsequent off-line analysis. All image acquisitions were performed by a well-trained sonographer and an investigator blinded to the study protocol performed all off-line analyses of the images. The analyses of the data were obtained by the Cardiovascular suite software (QUIPU srl, Pisa, Italy) Peak brachial FMD was expressed as percent increase in BAD from baseline. In order to assess the magnitude of the shear stimulus imposed in the various conditions, the peak wall shear rate (PWSR) was calculated as the ratio of 4 times the peak hyperemic blood velocity (cm/sec) to BAD (cm) [18]. PWSR provides an estimate of wall shear stress, assuming a constant hematocrit (blood viscosity). As previously reported, the coefficient of variation for FMD in our laboratory is 9% [17]. This estimate is based on measurements taken in the same individuals (n=40) on two different days, and thus includes an FMD reading error and biological variability.

Measurement of Intima-media thickness

Carotid B-mode ultrasound examination was performed in each subject using a validated protocol [19], with a 7.5-MHz multifrequency linear-array probe (Aplio XG imaging system, Toshiba, Japan), as previously described. Both the common and the internal carotids were scanned bilaterally by experienced vascular sonographers, blinded to the subject's clinical features. The probe was placed along the vessel axis and carotid arteries were explored with longitudinal (anterior, lateral and posterior) and transverse scan. The probe was manipulated so that the near and far walls were parallel to the probe, and the lumen diameter was maximized in the longitudinal plane. The origin of carotid bifurcation was identified and served as a reference point for the start of the measurements. IMT values were obtained at the far-wall on both side of the carotid bifurcation using a digital caliper and a semi-automated edge detection system (Toshiba), which provided the average thickness across 1- cm segment. The echographic images

were also stored according to DICOM standards for subsequent supervision and evaluation by a senior investigator blind of subject's clinical data or condition.

In *vitro* studies

Human coronary artery endothelial cells (HCAECs) were purchased from Cambrex Bio Science (Walkersville, MD, USA). Cells were grown in EGM 2 medium with endothelial cell growth supplement and 10% fetal serum (Cambrex Bio Science) and used at passages 2 to 5 [20]. Intracellular fluorescent probes diaminofluorescein diacetate (4-amino-5-methylamino-20,70-difluoro-fluorescein diacetate - DAF-FM DA, Invitrogen, USA) were used for flow cytometric detection of reactive nitrogen species produced by HCAECs. Approximately 0.5 x 10^6 cells/ml were incubated with 5 µl of DAF-FM DA (final concentration 10 µmol/l). All solutions were diluted in HBSS (Sigma Aldrich) in 3 ml FACS tubes and incubated in 37 °C water bath in the dark for 20 min. In blank samples, the fluorescent probes were replaced by HBSS. The reaction was stopped after 20 min, the samples were immediately measured in flow cytometer (FACS Calibur, Becton Dickinson, USA) using the Cell Quest software. For expression and statistical evaluation of results, the median of the positive DAF-FM DA peak subpopulations was evaluated [21]. For the studies in *vitro*, we used the same polyclonal IgG preparation used in *vivo* (see above).

Calculations and statistical analysis

HOMA-IR index was calculated as the product of fasting plasma glucose (mg/dl) and serum insulin concentration (mU/L) divided by 405 [22].

Arm (forearm and hand) blood flow (ABF) was obtained as the product of mean blood velocity by the transverse vessel area (obtained as πr^2) measured at baseline in the brachial artery before FMD measurements.

Patient sample size of the current protocol was calculated based on the expected changes in FMD following the IVIgG infusion. Assuming a significance level of 5% and 90% study power, a sample of 23 patients was sufficient to demonstrate an improvement in the primary variable (FMD) by 1 SD (5%). We recruited 24 patients for possible drop-out.

Data *in vivo* are expressed as mean±SEM. Statistical analysis was performed by paired or unpaired t test as appropriate (SPSS Inc., version 21.0, Chicago, IL). All data obtained after IVIgG infusion were compared only with baseline and Bonferroni correction was applied for multiple comparisons. A p value less than 0.05 was considered statistically significant.

RESULTS

The groups of control subjects and patients with CVID were similar for age (40±5 and 45±3 yrs), BMI (25.2±0.3 and 26±0.8 kg/m^2), heart rate (74±3 and 74±3 bpm), systolic blood pressure (123±2 and 119±3 mmHg), diastolic blood pressure (75±2 and 74±2 mmHg), total cholesterol (198±16 and 171±8 mg/dl), and fasting plasma glucose (74±3 and 77±3 mg/dl, respectively). As expected, compared to controls, circulating IgG were significantly reduced in the CVID patients (9.2±0.7 and 5.7±0.4 g/L, respectively, $p<0.05$).

IMT and **Endothelial function in *vivo*.**

Patients with CVID at baseline showed a reduced FMD compared to controls ($p<0.05$), while BAD, PWSR and NMD were similar in the two groups of subjects studied (Table I).

To evaluate the presence of initial signs of atherosclerosis, we measured the IMT of the carotid arteries in our subjects. IMT of the carotid arteries was similar in CVID patients (0.77±0.09 and 0.70±0.06 mm) or HC subjects (0.72±0.03 and 0.73±0.04 mm, in right and left carotid artery, respectively)..

To clarify whether IgG are involved in the regulation of vascular reactivity *in vivo* in humans, we studied the patients with CVID before and after the infusion of polyclonal IgG. As shown in figure 1, panel A and B, following the infusion of therapeutic polyclonal IgG, the levels of circulating plasma IgG rapidly normalized, whereas hs-CRP serum concentration dropped. From the baseline concentration of 5.8±0.4 g/L, plasma level of IgG raised to 10.8±0.7 g/L at day 1 ($p<0.001$), to slowly decrease in the following weeks (6.5±0.4 g/L at week 3, $p<0.001$ vs. baseline). Because of polyclonal IgG infusion, hs-CRP serum concentration decreased gradually from 5.9±1.5 mg/L to the *nadir* of 3.2±0.8 mg/L at week 1 ($p<0.05$ vs. baseline) to stay steady until the end of the study (3.3±1.1 mg/L, $p<0.05$ vs. baseline).

As shown in Figure 1, panel C, and Table I, therapeutic polyclonal IgG in CVID patients induced a marked and significant improvement of endothelial mediated vascular reactivity as FMD increased from 7.6±0.6% at baseline to 10.4±0.7% twenty-four hours after the infusion ($p<0.001$). FMD remained significantly higher than baseline until two weeks after the infusion of polyclonal IgG when, following the decrease of serum IgG concentration, dropped to 8.0±0.5 (p=NS vs. baseline). In table I we show the data relative to BAD, PWSR, and NMD in the control subjects and in patients with CVID. In the patients with CVID, PSWR remained unchanged after IVIgG. NMD was also unchanged. Compared to HC, basal ABF was significantly reduced at baseline in CVID patients and did not change during IVIgG infusion (Table I).

In protocol B, in seventeen CVID patients, endothelial mediated vascular reactivity was evaluated once more few weeks later. At variance than in protocol A, in this group of subjects, baseline evaluation was done 3 weeks after the previous IVIgG. In CVID patients, baseline FMD was significantly lower than HC (7.59±0.50 to 9.50±0.60%, respectively, $p=0.02$), but rose quickly and potently 1 hour after the IVIgG (9.08±0.55%, $p<0.005$ vs baseline, Fig. 1, panel D and table I). Again, BAD, PWSR, and NMD were similar in both groups of subjects studied and before and after the infusions (Table I).

Endothelial function in *vitro*.

To explore the possibility that the effects on endothelial function observed *in vivo* in CVID patients following the infusion of polyclonal IgG might be directly due to the interaction of the infused IgG with the endothelial cells, we exposed human coronary artery endothelial cells (HCAECs) to polyclonal IgG at physiological concentrations *in vitro*. In a first series of experiments HCAECs were treated with a wide range of concentration of poly IgG (33-133 μmol/L or 5-20 mg/ml) for 30 min, and NO producing cells were detected by flow cytometry

following staining of cells with DAF-FM. Control cells were treated with an isosmolar solution containing Bovine Serum Albumin (BSA). Figure 2, panel A, shows the results of multiple experiments demonstrating that poly IgG (33-133 μmol /L or 5-20 mg/ml) induced a significant increase (about 7-fold) in the number of NO-producing cell compared to control cells exposed to BSA ($p<0.005$). In a second series of experiments, we performed a time course of NO production in HCAECs treated with poly IgG (100 μmol/L or 15 mg/ml) or BSA (100 μmol/L or 6.65 mg/ml). Figure 2, panel B, shows that the number of HCAECc producing NO increased significantly beginning from 5 min after stimulation ($p<0.005$). The in *vitro* data indicate that IgG stimulation induces an increase in NO production by a direct action on endothelial cells.

Insulin-sensitivity.

In fig. 3, we report the relative changes of fasting plasma glucose (FPG) and serum insulin concentration following IVIgG (panel A and B). Only the data of the patients without diabetes were included in this analysis (n=21). Whereas FPG remained constant in the days and weeks following the IVIgG, serum insulin concentration significantly dropped from 9.31±1.17 to 4.40±0.49 μU/ml (at baseline and 3 weeks after IVIgG, respectively, $p<0.001$), suggesting a relevant change in insulin sensitivity due to the IVIgG. Consistently, the calculation of HOMA-IR index (see Fig. 3, panel C) confirmed a dramatic change in insulin sensitivity (from 1.82±0.28 to 0.87±0.10 $mg \cdot \mu U \cdot ml^{1}$, at baseline and 3 weeks after IVIgG, respectively, $p<0.001$). The 3 patients with diabetes were excluded from this analysis.

Discussion

We show that chronic reduction of circulating IgG is associated with impairment of endothelial mediated vascular reactivity and that such impairment can be corrected in the short term infusing i.v. polyclonal IgG. NO bioavailability *in vivo*, measured as FMD of brachial artery, increases rapidly one hour after the infusion of polyclonal IgG. In *vitro,* direct exposure of isolated endothelial cells to IgG is followed by an increase in NO release. Taken together, the data *in vivo* and in isolated arterial endothelial cells *in vitro* demonstrate that IgG directly stimulate endothelial cells and increase NO production. As ancillary observation, circulating IgG appear to be capable of affecting insulin action, as indicated by the powerful effect of polyclonal IgG infusion on circulating serum insulin concentration and whole-body insulin sensitivity. To the best of our knowledge, these data are the first demonstration that IgG exert a regulatory action in *vivo* on endothelial function and insulin sensitivity in humans.

The patients with CVID may represent an ideal model to study the effects of perturbation of circulating IgG on the endothelial function in vivo in humans. The reduction of circulating IgG might potentially trigger the atherosclerotic process since is associated with abnormal endothelial mediated vascular reactivity. IMT can be considered a marker of atherosclerosis and a consequence of impaired NO release [23, 24]. Nevertheless, in our patients, IMT of the carotid arteries was unaffected. Thus, we observe an impaired FMD, but a normal IMT. However, such contradiction is only apparent, since our CVID patients had been chronically treated with IVIgG for many years and the defect in circulating IgG had been corrected by this therapy. On the other hand, untreated patients with CVID do not appear to have an increased risk for CVD [1, 5, 14, 15], but rather they may show a sensible risk of infectious diseases or cancer. Such increases hierarchically do not allow the manifestation of CVD that requires long exposure to atherosclerotic risk factors, including endothelial dysfunction. However, the correction of IgG

deficiency by using the IVIgG corrects the defect reducing the risk of disease complications and improving mortality and morbidity in patients with CVID [14, 15].

The reduction of circulating insulin concentration and, consistently, the improvement of HOMA-IR index following the injection of IgG represents an ancillary intriguing finding of the current paper. For the calculation of HOMA-IR glucose and insulin concentrations in fasting state are used, when insulin-mediated skeletal muscle glucose uptake is minimally stimulated. However, solid evidences demonstrate that HOMA-IR is representative of insulin-mediated whole-body glucose uptake measured by more sophisticated techniques (25,26). FcγR have never been identified in skeletal muscle cells. Therefore, a direct action of IgG on skeletal muscle insulin mediated glucose disposal cannot be claimed. It has been hypothesized that insulin action can be improved by increasing the delivery of insulin and glucose to the skeletal muscle via an increased blood flow [27-29]. However, the IVIgG does not change the total blood flow to the periphery, as suggested by the fact that ABF is rather depressed in CVID patients and is unaffected by polyclonal IgG infusion (Table I). Nevertheless, it must be considered that total ABF might remain constant, but the relative distribution of blood within the regional, tissue vascular beds of the arm might have been changed [30]. In addition, the improvement of FMD observed after IVIgG is not consistent with the change in HOMA-IR, since FMD improves for one week and then returns close to baseline values by time 2-week, whereas HOMA-IR is constantly improving until 3 weeks. On the other hand, FMD measures endothelial function of the conduit arteries and an improvement of resistance vessel endothelial function, not evaluated in the current study, could be present. In our data, circulating IgG remain significantly higher than baseline after IVIgG at any time point, even when FMD has returned to baseline values (Fig. 1). Therefore, IgG might trigger additional, non-blood flow-mediated mechanisms capable and responsible of the improved insulin action observed in the current study. On this regard,

recent observations in mice have suggested that IgG may affect insulin sensitivity through a mechanism not involving changes in endothelial vasodilation or blood flow, but rather an improvement in the endothelial-mediated endocytosis process that favours the transit of insulin from the vessel lumen to the interstitial space and makes more insulin available for the peripheral tissues [13]. It is therefore possible that improved endocytosis in the endothelial cells might contribute to the increased availability of insulin for the skeletal muscle tissue [13].

The role played by CRP in the regulation of endothelial function has been extensively studied. It has been demonstrated in mice that CRP can inhibit insulin-induced eNOS activation and cause insulin resistance [9,11,12]. On the other hand, it has also been shown in isolated vessel in *vitro* that CRP can stimulate NO production and is not associated with endothelial dysfunction *in vivo* [31]. In the current study, CRP progressively, although not significantly, declines after the infusion of IgG and we cannot exclude that such reduction somehow might affect *in vivo* endothelial function or insulin sensitivity. However, from our data *in vitro* and from the data from protocol B, in which patients were studied one hour after IVIgG, we show that IgG can directly stimulate NO production in endothelial cells, indicating a direct role of circulating IgG in the regulation of endothelial function, sufficient and independent from the role potentially played by CRP. On the other hand, we cannot rule out that CRP changes might affect insulin sensitivity under these circumstances. Further studies are needed to clearly dissect the relative role of CRP and IgG on peripheral insulin sensitivity in humans.

In conclusion, our study indicates that circulating IgG play a direct role in the tonic regulation of endothelial function. In addition, the infusion of IgG is associated with relevant changes in insulin sensitivity *in vivo*. Whether these effects are involved in the pathogenesis of CVD remains to be elucidated and would need further studies.

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Table I. Brachial Artery Diameter (BAD) and Arm Blood Flow (ABD, before FMD measurement), Shear rate (PWSR), Flow Mediated Dilation (FMD), and Non-endothelial-Mediated Vasodilation (NMD) in the groups studied.

			IVIgG (Protocol A, n=24)				IVIgG (protocol B, n=17)	
Parameter	Study Group	Baseline	Day 1	Week 1	Week 2	Week 3	Baseline	1 Hour
BAD (mm)	CVID	3.64±0.13	3.62±0.13	3.61±0.12	3.62±0.11	3.64±0.13	3.51±0.14	3.55±0.14
	HC	3.75±0.13						
PWSR (sec^{-1})	CVID	91.5±6.6	95.6±5.4	92.1±4.8	102.6±6.6	95.6±5.5	93.9±7.9	88.7±8.8
	HC	93.0±6.3						
ABF (ml/min)	CVID	33.2±3.9*	39.3±5.4	29.8±2.9†	36.2±2.6	34.5±3.7	27.0±2.2†	46.3±14.2
	HC	47.9±6.5						
FMD (%)	CVID	7.6±0.6*	10.4±0.7‡	10.1±0.6‡	8.0±0.5	7.9±0.5	7.6±0.5*	9.8±0.5**
	HC	9.5±0.6						
NMD (%)	CVID	20.7±2.1	22.2±1.8	23.4±1.8	22.1±2.7	22.5±2.7	21.2±2.0	23.0±2.0
	HC	21.4±1.6						

*p<0.05 and †p<0.01 vs HC; **p<0.01 or ‡p<0,001 vs corresponding Baseline

Figure legend

Figure 1

Circulating plasma IgG (panel A), hs-CRP (panel B), and FMD of the brachial artery (panel C and D) in 24 patients with Common Variable Immunodeficiency before and after the i.v. infusion of polyclonal IgG at different time points. In panel D, FMD after IVIgG in 17 patients with CVID. Data are expressed as M±SEM. †p<0.005, and ‡p<0.001 vs. appropriate baseline values.

Figure 2.

NO producing human coronary artery endothelial cells (HCAECs) detected by flow cytometry following staining of cells with DAF-FM in response to polyclonal IgG (solid line) or Bovine Serum Albumin (BSA, broken line) at different concentration (panel A) or time point (panel B). Data are expressed as M±SD and represent the result of 3 experiments for each panel. **p<0.005 vs Bovine Serum Albumin treated cells.

Figure 3.

Fasting plasma glucose (panel A), serum insulin (panel B), and HOMA-IR index (panel C) in 21 patients with Common Variable Immunodeficiency before and after the i.v. infusion of polyclonal IgG at different time points. Data are expressed as M±SEM *p<0.05, †p<0.01 and ‡p<0.002 vs. baseline values.

Fig. 1

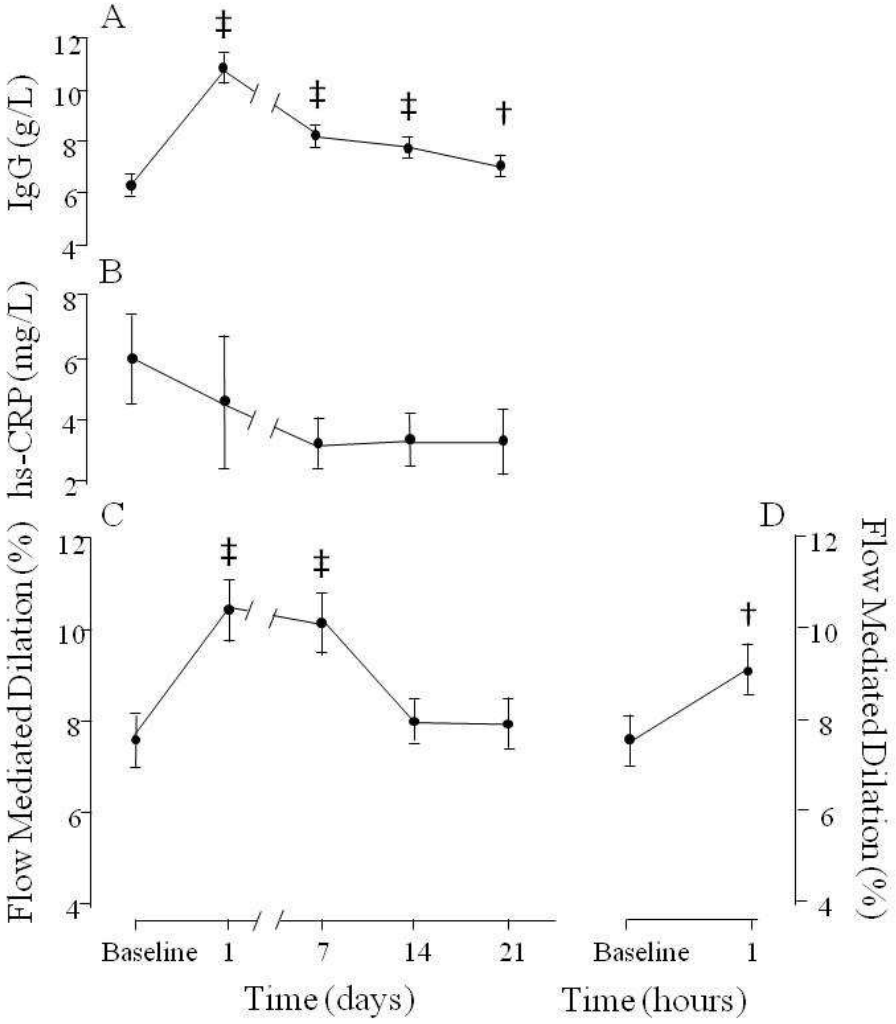
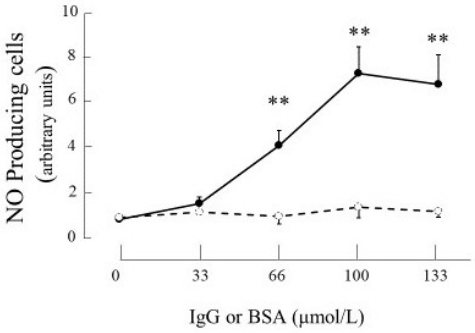

A
IgG (g/L)
12
10
8
6
4
‡
‡
‡
†
B
hs-CRP (mg/L)
8
6
4
2
C
Flow Mediated Dilation (%)
12
10
8
6
4
‡
‡
D
†
Flow Mediated Dilation (%)
12
10
8
6
4
Baseline
1
7
14
21
Time (days)
Baseline
1
Time (hours)

Fig. 2

A



B

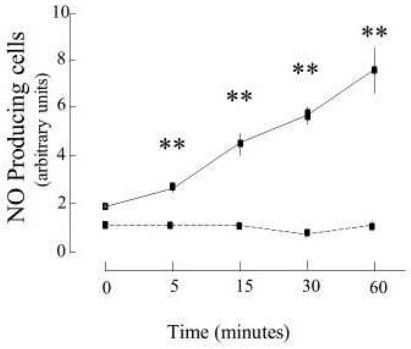
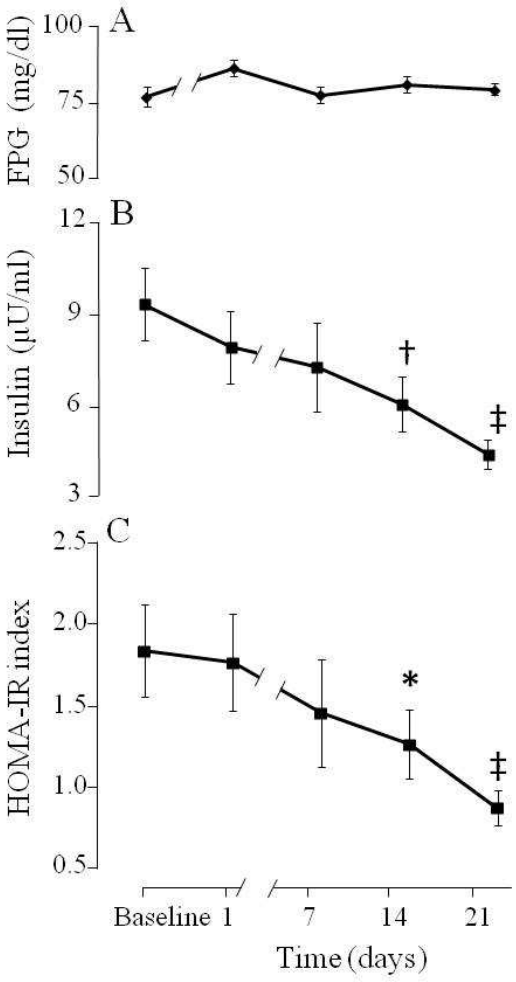


Fig.3


A
FPG (mg/dl)
100
75
50
B
Insulin (μU/ml)
12
9
6
3
†
‡
C
HOMA-IR index
2.5
2.0
1.5
1.0
0.5
*
‡
Baseline 1
7
14
21
Time (days)

Highlights

- Patients with CVID have a depressed endothelial function.
- Infusion of polyclonal IgG rapidly corrects endothelial dysfunction in vivo
- In vitro, exposure of isolated endothelial cells to IgG induces NO production.
- IgG infusion in CVID patients improves insulin sensitivity.

- In vivo in humans that IgG can affect endothelial mediated nitric oxide bioavailability and play a role in the regulation of insulin sensitivity

- Circulating IgG might exert a tonic role in the maintenance of a healthy vascular bed by stimulating the release of nitric oxide and potentially play an anti-atherogenic role in humans and affect insulin sensitivity.

From biodegradable polymers to bioresorbable vascular scaffolds: Available evidence in the current era of new-generation DES

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Abstract

New-generation drug-eluting stents (DES) encompass a large variety of coronary devices, featuring thin struts, biocompatible durable or biodegradable polymer coatings, and limus-eluting drugs. Due to improved early and long-term outcomes among patients undergoing percutaneous coronary intervention, new-generation metallic DES are recommended in almost all patient and lesion subsets. Available evidence from randomized trials indicate a similar safety and efficacy profile between biodegradable and durable polymers new-generation DES. Recently, polymer-free DES provided promising results particularly as alternative to bare-metal stents. Ultimately, although remaining conceptually solid, bioresorbable vascular scaffolds represent an immature technology owing to increased risk of thrombosis. In this review, we summarized current evidence about contemporary coronary devices.

Introduction

In 2017, percutaneous coronary intervention (PCI) has entered its 40th anniversary since the first description of balloon coronary angioplasty by Andreas Grüntzig in 1977. Since then, the safety and efficacy of the procedure has continuously improved with advances in the technique, equipment, and procedural success. In the late 1980s, the advent of metallic coronary stents (BMS) favored the widespread adoption of PCI in routine clinical practice by eliminating the risk of abrupt vessel closure as well as the need for standby emergent coronary artery bypass grafting (CABG). Nowadays, PCI constitutes the most commonly performed therapeutic procedure in the field of cardiology and one of the most common interventions performed in medicine.

Stent implantation has become the treatment of choice for patients with coronary artery disease since the late '90s. With steady ameliorations in stent deliverability and reductions in acute stent thrombosis, the first limitation of BMS became in-stent restenosis, leading to the introduction of drug-eluting stents (DES). These new devices shared similar mechanical

advantages of BMS with the advantage of delivering an antirestenotic drug to the arterial wall, decreasing rates of restenosis. Unfortunately,, their use was associated with an increased risk of late (>1-year) thrombotic events.(1-3) New-generation DES entailed a broad range of refinements affecting the antiproliferative drug, metallic stent platform, and the polymer coating.(4)

While the antiproliferative drug paclitaxel has been widely used in the past, it has been largely replaced by analogues of rapamycin ("limus family") that inhibit the mammalian target of rapamycin (mTOR) and its two related complexes (mTORC1 and mTORC2), are associated with a broader therapeutic window, inducing reversible arrest of cell cycle at the G_1-S transition phase

Concerning stent platform, new-generation DES are usually made of cobalt chromium or platinum chromium, which allowed for a 40-50% decrease in strut thickness (from 130-140 to 60-80 μm) while maintaining radial strength, providing sufficient radio-opacity, and reduced nickel content. The potential advantages of coronary stents featuring thinner struts

are not only related to procedural aspects but also to faster and better endothelial coverage after stent implantation.

Polymer coatings, applied to the stent surface to control drug delivery over time, have been associated with chronic inflammation, and, in some cases, with extensive hypersensitivity reactions(5). Poor healing and delayed endothelialization related to permanent polymer in DES might associated with very late stent-thrombosis, thus prompting further iteration in the field, resulting in more biocompatible permanent polymers and biodegradable polymers, or even in the elimination of polymer coatings from stent surface.

Bioresorbable vascular scaffolds (BRS) represent a further iteration in coronary device technology by affording a transient support to the coronary vessel for a variable period ranging from as short as 1 year to several years according to scaffold composition.

This review provides a summaryabout newer generation coronary devices by summarizing the evidence from randomized trials and outlining future perspectives on coronary stent technology.

Biodegradable Polymers DES

Biodegradable polymers (BP) are temporary coatings that, after drug release, are cleared from the stent surface over a variable period of time. Polyacidic acid copolymers, such as the polylactic acid (PLLA) or polyglycolic acid, are commonly adopted in this DES technology and biodegradation occurs via a hydrolytic process. **Figure 1** shows the structure and composition of biodegradable polymer DES.

Biolimus-eluting stents. Biolimus-eluting stents (BES) include the BioMatrix BES (Biosensors, Morges, Switzerland) and Nobori BES (Terumo, Tokyo, Japan). Although both BES were initially made of stainless steel and most data are derived from this platform, the BioMatrix alpha has a cobalt chromium platform with a thin strut backbone. Biolimus is eluted only from the abluminal matrix containing PLLA (15.6 μg each 1 mm stent length in 1:1 ratio), which is metabolized via the Krebs cycle into CO_2 and water after 6-9 months. The main trials that have investigated the performance of the BioMatrix BES are the LEADERS (vs. Cypher sirolimus-eluting stent

[SES]; n=1,707),(6) COMFORTABLE-AMI (vs. Gazelle BMS in patients with acute myocardial infarction; n=1,161),(7) and SORT-OUT VI (vs. Resolute Integrity zotarolimus-eluting stent [ZES]; n=2,999) trials.(8) Collectively, these trials showed a greater safety profile of the BioMatrix BES compared with BMS and Cypher SES,(6,7) a greater efficacy compared with BMS,(7) and a similar safety and efficacy profile compared with R-ZES.(8) Two recent real-world registries confirmed the safety and the efficacy of Biomatrix BES.,(9,10) also when compared with a second generation DES such as the Xience Prime.(10) However, a small randomized study, comparing strut coverage at 9-month optical coherence tomography (OCT) between the Biomatrix BES and the Xience Prime everolimus-eluting stents (EES), showed a higher proportion of uncovered struts with BP-BES.(11)

The Nobori BES presents a stainless steel structure with strut thickness of 120 μm. The abluminal surface is coated with PLA, which degrades in the same way and time as the Biomatrix stent. A non-biodegradable basecoat of parylene C (2 μm) is additionally present.(12,13) The clinical performance of the

Nobori BES has been evaluated in 4 trials: the SORT-OUT V (vs. Cypher SES; n=2,468),(14) COMPARE II (vs. Xience V or Xience Prime EES; n=2,707),(15) NEXT (vs. Xience V or Xience Prime EES; n=3,235),(16) and SORT-OUT VII (vs. Orsiro SES; n=2,525) trials.(17) These studies reported similar efficacy and safety outcomes of the Nobori BES compared with Cypher SES, Xience EES and Orsiro SES.(14,15) However, in the SORT-OUT V trial, stent thrombosis, particularly within the first month after PCI, occurred more frequently after BES (0.7% vs. 0.2%, P=0.03).(14) Along the same vein, in the SORT-OUT VII, the rate of definite stent thrombosis was significantly higher in patients randomized to the BP-BES group compared with the Orsiro SES (1.2% vs. 0.4%, P=0.034), mainly driven by an excess of early thrombotic events (0.8% vs. 0.2%, P=0.038). However, no statistically significant differences were observed in terms of overall definite or probable stent thrombosis between the two groups at 12-month follow-up.(17) A recent randomized trial, reported the non-inferiority of a short-duration dual antiplatelet therapy (6 months) in comparison with a more prolonged regimen (18 months) after implantation of the Nobori

BP-BES.(18) Finally, the long-term follow-up of the NOBORI 2 registry (n=3,067) showed a good performance of this device in terms of both safety and efficacy outcomes at 5 years.(19)

Orsiro SES. The Orsiro BP-SES (Biotronik AG, Bülach, Switzerland) combines a biodegradable PLLA polymer with an ultra-thin strut cobalt chromium platform (60 µm for stent diameters up to 3.0 mm, 80 µm for stent diameters >3.0 mm). Approximately 80% of sirolimus is eluted over a period of 100 days, while the polymer degrades over a period of 14 months. The polymer matrix has an asymmetric design that allows for the release of a greater drug dose on the abluminal than luminal side. Five randomized trials have investigated the efficacy and safety of Orsiro SES: the BIOFLOW-II (vs. Xience Prime EES; n =452),(20) BIOSCIENCE (vs. Xience Prime EES; n =2,119),(21) SORT OUT VII (vs. BP-BES; n=2,525, see above),(17) BIORESORT (vs. Resolute Integrity ZES or Synergy BP-EES; n=3,514),(22) and the BIOFLOW-V (vs. Xience Prime EES; n=1,334)(23) trials. Collectively, these trials demonstrated a similar angiographic performance compared with EES,(20) and similar clinical safety and efficacy compared

with EES,(24) Nobori BES(17) and R-ZES.(25) While clinical outcomes have been similar between the Orsiro SES and the Xience EES among diabetic patients,(26) a possible superiority of the Orsiro SES has been suggested among patients with acute myocardial infarction.(27) Moreover, the BIOFLOW-V trial reported a lower risk for target-vessel myocardial infarction with the Orsiro SES than EES.(28) Of interest, a low thrombogenicity with the Orsiro SES has been found in a network meta-analysis of 147 trials (n=126,526) showing a lower risk of stent thrombosis at 1-year with the Orsiro SES compared with Cypher SES and BES.(29)

Synergy EES. The Synergy stent (Boston Scientific Corporation, Marlborough, MA, USA) is a thin-strut (74-81 μm), platinum chromium metal alloy platform, with an abluminal PLGA polymer, eluting everolimus (100 μg/cm^2). Drug elution and polymer biodegradation occur within 3-4 months.(30,31) In a multicenter, single-arm study, strut coverage of the entire stent was almost complete at 3-month after stent implantation.(30) Four randomized trials have assessed the angiographic and clinical performance of the

Synergy EES: the EVOLVE I (vs. Promus Element EES; n=291),(32) EVOLVE II (vs. Promus Element EES; n=1,684),(33) EVOLVE CHINA (vs. Promus Element EES; n=412),(34) and BIORESORT (n=3,514) trials.(25) These trials showed similar angiographic results of the Synergy EES compared with the Promus EES, and a similar safety and efficacy profile compared with the Promus EES and the R-ZES.(25,32,33) Comparable safety and efficacy outcomes between the Synergy EES and other new-generation DES in a real-world scenario have been confirmed by two recent registries.(35,36) Recently, the SENIOR trial compared the Synergy EES against the Rebel BMS (Boston Scientific) among 1,200 patients aged 75 years or more on a background dual antiplatelet therapy administered for 1 to 6 months. At 12-month follow-up, the primary endpoint, a composite of all-cause mortality, myocardial infarction, stroke, or ischemia-driven target vessel revascularization, occurred in 11.6% of patients randomly allocated to Synergy EES and in 16.4% of patients randomly assigned to BMS (RR 0.71, 95%CI 0.52-0.94).(37)

Ultimaster SES. The Ultimaster SES (Terumo Corporation, Tokyo, Japan) is made of a cobalt-chromium with thin struts (80 μm) and a biodegradable polymer (PDLLA and polycaprolactone) applied to the abluminal side. Sirolimus (3.9 μg/mm) is eluted over a period of 3 months. In addition, the Ultimaster SES features a gradient coating, i.e. the polymer coating is absent on the stent areas experiencing the highest physical stress, which may reduce the risk of polymer cracking and delamination.(38) The CENTURY II trial (n=1,119) showed the non-inferiority of Ultimaster SES compared with Xience EES.(39)

Tivoli SES. The Tivoli SES (EssenTech, Beijing, China) is a thin-strut (80 μm), cobalt chromium metal platform with a PLGA polymer, eluting sirolimus (8 μg/mm). Approximately 75% of the sirolimus is eluted at 30 days. The I-LOVE-IT 2 trial (n=2,737) reported the non-inferiority between the Tivoli SES and a durable polymer first generation DES, the Firebird SES.(40) A subsequent analysis of the I-LOVE-IT trial assessed noninferiority in safety and efficacy of 6-month versus 12-

month dual antiplatelet therapy after implantation of Tivoli SES, with similar event rates in both groups.(41)

MiStent SES. The MiStent (Micell Technologies, Durham, NC, USA) is cobalt chromium, ultra-thin strut (64 μm), PLGA-based, sirolimus-eluting stent. PLGA carries a crystalline form of sirolimus. The PLGA/sirolimus combination is eliminated from the stent surface within 45-60 days and PLGA is fully degraded within 90 days. Crystalline sirolimus remains in the tissue and continues to elute the drug into the surrounding tissue for up to 9 months. The DESSOLVE II trial (n=180) reported superiority for in-stent late lumen loss at 9 months for the MiStent compared with Endeavor ZES.(42) The ongoing DESSOLVE III trial, which has enrolled about 1,404 patients in 20 sites in Europe, will provide further clinical data of this device (ClinicalTrials.gov identifier: NCT02385279).

Combo SES. The Combo stent (OrbusNeich Medical, Ft. Lauderdale, FL, USA) is a 100 μm thick stainless steel stent covered abluminally with a biodegradable polymer matrix (PDLLA and PLGA), allowing for a controlled release of sirolimus (5 μg/mm). Sirolimus release is completed in 30 days

and the polymer coating is degraded within 90 days. An additional circumferential layer of anti-CD34 antibodies is applied on the stent struts on top of the polymer aiming to accelerate endothelial coverage. In the REMEDEE trial, the Combo SES was found non-inferior to the Taxus PES for the primary endpoint of in-stent late loss at 9-month.(43) The REMEDEE post-market registry (n=1,000) reported favorable results at 1-year, with a rate of stent thrombosis of 0.5% and no events beyond the periprocedural period.(44) Recently the results of two trials investigating the use of Combo SES have been reported. In the HARMONEE trial (n=572), the Combo SES was noninferior to Xience EES in terms of target vessel failure, even though event rate was higher in the experimental than control group (7% vs. 4.2%, respectively). In the REDUCE trial, a short dual antiplatelet therapy for 3 months resulted non-inferior to a standard 12-month regimen with respect to a composite safety and efficacy outcome among 1,496 patients treated with the COMBO stent.(45) However, stent thrombosis was numerically higher in the experimental than control group (1.2% vs. 0.4%; P=0.08).(45)

DESyne BD. The DESyne (Elixir Medical, Sunnyvale, CA, USA) is a cobalt chromium, thin strut (80 μm), PBMA-based, novolimus-eluting stent (NES). Novolimus that is a metabolite of sirolimus requires lower drug concentration (5 μg/mm) and less polymer coating (3 μm). Clinical data are limited to the EXCELLA II trial (n=210) demonstrated a greater angiographic efficacy compared with Endeavor ZES and favorable clinical outcomes, with a not-significant trend towards reduction in myocardial infarction and repeat revascularization in the NES group at five-year follow-up.(46)Compared with the DESyne, the DESyne BD (Elixir Medical, Sunnyvale, CA, USA) presents a PDLLA polymer, which is degraded over a period of 6-9 months. In the EXCELLA-BD trial (n=146), the DESyne BD showed a greater angiographic efficacy compared with the Endeavor ZES.(47)

Non-polymeric metallic DES

The concept of an antiproliferative drug directly applied to the stent surface without polymer coating represents a fascinating, natural evolution of existing approaches. Polymer-

free DES have been developed through both physical and chemical methods, mainly consisting of creating pores and reservoirs or by coating the stent with a porous inorganic material. The issue is to obtaining a correct drug load with a controlled and prolonged release; in fact, a very fast drug release has hampered initial efforts.

Biofreedom BES. The Biofreedom BES (Biosensors International Pte Ltd, Singapore) is made of stainless steel (112 μm), with the abluminal surface coated with biolimus by a microstructured, polymer-free structure. Approximately 98% of biolimus is released from the stent at 28 days. In view of such properties, this stent has been specifically tested for the treatment of patients at high bleeding risk to allow early discontinuation of dual antiplatelet therapy. In the LEADERS FREE trial, 2,466 patients deemed at high-risk for bleeding were randomized to the Biofreedom BES or the Gazelle BMS and received dual antiplatelet therapy for one month. Advanced age (≥75 years) and indication for oral anticoagulation after PCI were the two most frequent criteria qualifying for high-risk bleeding status. The trial reported a greater efficacy (clinically

driven target-lesion revascularization) and safety (cardiac death, myocardial infarction, or stent thrombosis) in favor of the Biofreedom BES at 2-year(48): the rate of the primary efficacy endpoint was 4.9% vs. 9.3% at 1-year (P<0.001) and 6.8% vs. 12% at 2-year (P<0.001), while the rate of the primary safety endpoint amounted to 9.2% vs. 12.7% (P=0.006) at 1-year and 12.6% vs. 15.3% at 2-year (P=0.039).(48) As a result, the Biofreedom BES may be particularly indicated for patients at high bleeding risk as well as for those with planned noncardiac surgery. The performance of this new generation BES in real-world patients with ST-segment elevation myocardial infarction has been recently evaluated in a registry involving 176 patients, suggesting acceptable clinical and angiographic results with only three (1.7%) target vessel revascularizations.(49) It is important to highlight that Biofreedom BES has been compared so far against BMS. In this context, early in-stent restenosis after stenting with Biofreedom BES has been reported in a small series and may require additional evaluatione.(50)

Yukon SES. The Yukon SES (Translumina, GmbH, Hechingen, Germany) presents a specifically designed surface

with micropores (2 μm deep) wherein the antiproliferative agent sirolimus is deposited. The platform is stainless steel in the Yukon Choice PC and cobalt chromium in the Yukon Choice Flex. The first randomized study on this device (LIPSIA Yukon Trial) compared Yukon Choice PC with Endeavor Resolute ZES in 240 diabetic patients and failed to show noninferiority with regard to the primary endpoint (in-stent late lumen loss) after a follow-up of nine months, without significant differences in clinical outcomes, which were confirmed at 5-year follow-up.(51) In the ISAR-TEST (n=450), Yukon Choice PC SES was compared with the permanent polymer paclitaxel-eluting Taxus stent; no significant differences in clinical outcome were observed, with a notably low incidence of ST (0.5%) in the polymer-free group;(52) however, data concerning the rates of 1-year target-lesion revascularization were discouraging (13.7% vs 4.4%, $P<0.05$).(53) A pooled analysis of the ISAR-TEST and LIPSIA Yukon trials (n =682) comparing polymer-free Yukon SES with PES reported a similar in-stent late loss and similar clinical outcomes.(54) In the ISAR-TEST 4 (n=2,603), the Yukon Choice PC SES was compared with the first-generation

permanent polymer Cypher SES and with the Xience EES, with comparable clinical outcomes at five years among the three group but with higher rates of device related events in the Cypher group.(55) The rates of stent thrombosis of Yukon Choice PC SES and Xience EES by the end of the study were comparable (1.2% vs. 1.4%, P=0.67%).(55) A real-world registry allowing unrestricted use of Yukon Choice PC SES (n=701), with a 5-year follow-up, reported low rates of stent thrombosis (1.14%, with a rate of very late events of 0.29%), but a remarkable high rate of target-vessel revascularization (23.5%).(56) The Yukon Choice Flex has been tested in an all-comers registry involving 778 patients.(57) The study reported an acceptable safety and efficacy profile at 1-year follow-up, with low rates of death, myocardial infarction, definite stent thrombosis and ischemia-driven target-lesion revascularization (2.4%, 1.9%, 0.3%, and 11.3%, respectively).(57)

Cre8. The Cre8 (Alvimedica, Istanbul, Turkey) is made of a cobalt chromium alloy (80-μm) and has an ultrathin (0.3 μm) passive carbon coating. The antiproliferative drug (sirolimus, 90 $\mu g/cm^2$) is formulated with a mixture of long-

chain fatty acids (amphilimus) to act as a carrier and control drug release (70% released within 30 days with a complete elution by 90 days). The drug is loaded into reservoirs on the abluminal surface. In the NEXT trial (n=323), the Cre8 stent had a greater angiographic efficacy than the Taxus PES,(58) and, in the RESERVOIR trial (n=112), was non-inferior to EES for neointimal volume obstruction among patients with diabetes mellitus.(59) In a propensity matching comparison between 187 patients treated with Cre8 and 150 patients treated with Xience EES, there was no significant difference at 1-year in terms major adverse cardiovascular events (7.4% Cre8 vs. 10.2% EES, P=0.261), all-cause mortality (1.3% Cre8 vs. 1.4% EES, P=0.823), target-vessel revascularization (5.2% Cre8 vs. 8.8% EES, P = 0.169).(60) In another registry involving about 1,200 patients treated with the Cre8, no significant difference in the rates of 1-year target vessel failure was observed in patients treated with a shortened dual antiplatelet therapy (3 months) in comparison with a standard duration (6 months or more).(61)

DFS. The drug-filled stent (DFS, Medtronic, Santa Rosa, CA, USA) is a novel polymer-free DES technology that features

a tri-layer wire design, with the inner layer removed to create a lumen continuously coated with sirolimus (1.1 μg/mm^2), which is released through multiple laser-drilled holes on the abluminal side of the stent. The middle layer is tantalum, which is required for radiopacity, and the outer layer is of cobalt chromium (81 μm). In the RevElution study, the DFS was non-inferior to R-ZES for in-stent late lumen loss and showed excellent strut coverage already at 1-month.(62)

Bioresorbable vascular scaffolds

Bioresorbable vascular scaffolds (BRS) were conceived to overcome the limitations of permanent metallic stent platforms by providing transient vessel support and local drug delivery followed by complete resorption in a variable time period (ranging from three to 42 months). The first BRS was developed in 1980's at Duke University.(63) Prototypical devices can have polymeric or metallic platform. PLLA is the most commonly used polymer and its degradation occurs through hydrolysis and depolymerization and provides substrates for the Krebs cycle. Metal-based scaffolds are usually

made of alloy combining magnesium or iron and rare earth metals to increase radial strength. Magnesium degradation occurs by corrosion and produces soft amorphous hydrohyapatite. Other materials for BRS include tyrosine polycarbonate or poly-lactic anhydride. Polymer or metal structure act as backbone for drug elution.

The first BRS implanted in human has been the Japanese Igaki-Tamai scaffold (Kyoto Medical Planning Co., Ltd., Kyoto, Japan) a PLLA-based, non-drug eluting self-expandable scaffold. For its initial expansion, the contrast needed to be heated up to 80 °C and applied through the delivery balloon; the final expansion was achieved at body temperature after 20–30 minutes from implantation.(64) The scaffold needed 18–24 months to fully disappear. A pilot study (19 lesions in 15 patients treated with 25 scaffolds) provided encouraging clinic and angiographic data.(64) In a second study of 50 elective patients (63 lesions, 84 stents), the 10-year follow-up showed a freedom from cardiac death, non-cardiac death, and major cardiovascular events of 98%, 87%, and 48%, respectively,(65) with a rate of target-lesion revascularization of 28% and two

definite stent thromboses.(65) However, the use of heat to induce self-expansion, the potentially injury for arterial wall, and the need for a 8-Fr guiding catheter led to abandon this early BRS.

Absorb BVS (Abbott Vascular, Santa Clara, CA, USA). The currently available version (1.1) of this device was developed to overcome some limitations of the first iteration (1.0). Its design, featuring in-phase hoops and direct links, allows a more uniform vessel wall scaffolding. The PLLA matrix is coated with a 1:1 mixture of PDLLA and everolimus (8.2 μg/mm) and confers a strut thickness of 150 μm. Full resorption of the scaffold needs 24-48 months. Slower hydrolysis of the 1.1 version in comparison with the first one achieves more prolonged radial support (6 months rather than weeks), in view to avoid an excess in lumen area reduction and late recoil observed in the first evaluation in humans of the 1.0 device.(66) The crossing profile is 1.4 mm and the only radiopaque components are the two platinum markers at the edge. The Cohort B trial, the first in man evaluation of the 1.1 Absorb BVS, provided favorable angiographic results and

interesting data concerning restoration of vasomotion at 1 and 3 years after BRS implantation.(67,68)

Magmaris (Biotronik AG, Bülach, Switzerland). Magnesium represents an essential component of many human enzymes and a co-factor for ATPase. The balloon-expandable Absorbable Metal Stent (AMS-1) (Biotronik, Berlin, Germany) was the first metallic BRS, and showed a sufficient radial strength, low elastic recoil (<8%), high collapse pressure (0.8 bar) and minimal shortening after inflation (<5%), but also unacceptable high rates of target-lesions revascularization similar to balloon angioplasty.(69)

The Magmaris (previously named DREAMS-2) is made of a magnesium alloy and a matrix of PLLA and sirolimus amounting to a strut thickness of 150 μm (120 μm for 2.5 mm diameter devices), with a 1.5 mm crossing profile requiring 6 Fr guiding catheter. The BIOSOLVE II (First in Man Study of the DREAMS 2nd Generation Drug Eluting Absorbable Metal Scaffold) study enrolled 123 patients with up two *de novo* lesions with a reference vessel diameter between 2.2 and 3.7 mm. At 6-month follow-up angiography, in-segment late lumen

loss was 0.27±0.37 mm, with 80% of patients showing evidence for restoration of vasomotion. At 12 months, a second angiographic follow-up was performed in 42 patients and mean paired in-segment late lumen loss at 6 and 12 months was 0.20±0.21 mm and 0.25±0.22 mm (P=0.12). The 1-year rate of target-vessel and target-lesion revascularization was 3.4% and 1.7%, respectively.(70) The performance of Magmaris is under further evaluation in the ongoing BIOSOLVE III and BIOSOLVE IV studies and in the Magnesium 1,000 Program registry. Preclinical observations of low thrombogenicity(71) and improved trackability(72) of Magmaris BRS are of interest but require further assessment.

DESolve BRS (Elixir Medical Corporation, Sunnyvale, CA, USA). The DESolve Novolimus-Eluting Bioresorbable Coronary Scaffold System is a poly-L-lactic-acid based polymer, novolimus-eluting scaffold. The first version of this device has been replaced by the DESolve Nx (150 μm strut thickness, 1.44 mm crossing profile, 6-Fr compatible) eluting novolimus at a dose of 5 μm/mm scaffold length. A small single-arm registry, the DESolve Nx study, reported initial data

on the performance of the DESolve Nx among 122 patients with *de novo* coronary lesions. Early lumen gain at 6-month was sustained through 24 months with a relatively low (7.4%) overall rate of major adverse events including cardiac death, target-vessel myocardial infarction, or clinically indicated target-lesion revascularization and one probable scaffold thrombosis within the first month.(73) A post-market registry, designed to assess long-term safety and performance of the DESolve NX in patients with stable coronary disease (n=102) and non-complex lesion (exclusion of lesions with excessive tortuosity, angulation, and moderate or heavy calcification) reported an overall major adverse cardiac events rate of 2% at 6-month follow up. The DESolve Cx and DESolve 100 represent further iterations of the device with modified stent features (120 and 100 μm) and novel delivery systems. A platform for specific use in patients with acute myocardial infarction (AMITY) is also under early clinical investigation.

Amaranth BRS (Amaranth Medical Inc, Mountain View, CA, USA). The Amaranth scaffold family is made a sirolimus-eluting BRSs with an ultra-high molecular weight

resin with an amorphous structure, with a radial force comparable to DES and with high fracture resistance and over-expansion capabilities: these scaffolds are able to tolerate up to 2.5 mm over nominal diameter.(74) In-vitro studies demonstrated that reduction in scaffold mass reaches ≈50% of the initial molecular weight at 8 months and >85% at 18 months.(75) A 4-year experience overall, with 2-year human results, has supported both biocompatibility and biomechanical sustainability.(74) Clinical program led to a progressive reduction in strut thickness (from 150 μm to 115 μm and an further improvement with a sub-100 μm scaffold).(74) The sirolimus-eluting version of the first scaffold (150 μm) was evaluated in the RENASCENT-I trial (n=63) providing good angiographic data (in-scaffold late lumen loss of 0.27±0.41 mm and binary restenosis rate of 1.6%), and an incidence of target-lesion failure of 4.9% at 9-month follow-up, with no case of stent thrombosis.(74) The second version of the device was evaluated in the RENASCENT-II trial (n=60) reporting high clinical success (98.3%), a low rate of major adverse cardiac events (3.4%), and no angiographic restenosis or stent

thrombosis.(74) The RENASCENT-III trial (n=57) will report results after implantation of the sub-100 μm scaffold.(74)

Future perspectives on BRS. Reduced strut thickness, higher radial force and biomechanical stability are the main features of novel BRS. New polymer-based scaffolds under clinical investigation include the MeRes100 (Meril Life Sciences, Vapi, Gujarat, India), the Mirage BRMS (Manli Cardiology Singapore), the ART (Arterial Remodeling Technologies; Noisy le Roi, France), and the Xinosorb (Huaan Biotechnology Co., Ltd., Hangzhou, Zhejiang, People's Republic of China). Non polymeric scaffolds are the Fantom (Reva Medical, San Diego, CA, USA) made of tyrosine polycarbonate, the Ideal BioStent (Xenogenics Corp, Canton, MA, USA) that contains poly-lactic anhydride and the Acute BRS (OrbusNeich, Fort Lauderdale, FL, USA). Novel metallic scaffolds are also being examined: the FADES (Zorion Medical, Indianapolis, IN, USA), the Medtronic Mg Absorbable Scaffold, BSCI Mg Absorbable Scaffold and QualiMed Mg Absorbable Scaffold.

Clinical trials comparing BRS vs. New-DES

So far, the Absorb BVS has been the only BRS evaluated in a randomized clinical setting. Currently, 8 randomized trials have compared the Absorb BVS 1.1 with new-generation DES, predominantly Xience EES: ABSORB China (n =480),(76) ABSORB II (n =501),(77) ABSORB III (n =2,008),(78) ABSORB IV (n=2,604),(79) ABSORB Japan (n =400),(80) AIDA (n=1,845),(81) EVERBIO II (n =158),(82) TROFI II (n=191).(83)

The ABSORB CHINA enrolled patients undergoing elective PCI with a maximum of 2 de novo coronary artery lesions with reference vessel diameter 2.5 to 3.75 mm and length ≤24 mm. BVS was non-inferior to EES with respect to the primary angiographic endpoint of in-segment late loss and afforded similar rates of target-lesion failure and device thrombosis.(76) In the ABSORB II trial, which enrolled patients undergoing PCI for up to 2 de novo native coronary artery lesions, each located in different major epicardial vessels, with an angiographic maximal luminal diameter between 2.25 and 3.8 mm and a lesion length of≤48 mm, the co-primary endpoint

of angiographic vasomotor reactivity was not improved among patients treated with Absorb BVS (superiority hypothesis) and the co-primary endpoint of late lumen loss was not non-inferior compared with the EES (non-inferiority hypothesis) at 3 years follow-up.(77) The minimum lumen diameter was significantly smaller in the Absorb BVS compared with EES group and the percentage diameter stenosis was higher. In terms of clinical outcomes, the 3-year rates of myocardial infarction, device-oriented composite endpoint, and definite or probable device thrombosis were higher in the Absorb BVS compared with the EES group.(77) At 4-year follow-up, target-lesion revascularization was somewhat higher with the Absorb BVS (11.1% vs. 5.6%, respectively, P=0.05) and the cumulative rate of definite or probable scaffold thrombosis amounted to 2.8%. At 2 years, a higher rate of target-lesion failure among patients treated with Absorb BVS compared with EES was observed in the ABSORB Japan trial, in which there were 4 cases of very late scaffold thrombosis (1.6%) and no very late thrombosis was observed in the DES cohort.(84) In a meta-analysis of randomized trials and observational studies, the Absorb BVS

was associated with higher risk of very late scaffold thrombosis compared with EES and a numerically increased risk of target-lesion failure.(85) The Absorb III trial has been a larger, multicentre randomized trial comparing Absorb BVS with Xience EES in patients with stable or unstable coronary artery disease and one or two new native coronary artery lesions in separate epicardial coronary vessels to be no more than 24 mm in length with a reference-vessel diameter of 2.5 to 3.75 mm on visual assessment, with exclusion of patients with acute myocardial infarction and longer or complex lesions. The study was designed and powered to assess clinical non-inferiority of Absorb BVS in comparison with Xience EES, with a primary endpoint of target-lesion failure defined as combination of cardiac death, target-vessel myocardial infarction, or ischemia-driven target-lesion revascularization at 1 year. The primary endpoint occurred in 7.8% of patients randomized to the Absorb BVS and in 6.1% of patients randomized to the Xience EES (P=0.007 for noninferiority), without significant difference in target-vessel myocardial infarction (6.0% and 4.6%, respectively, P=0.18), or ischemia-driven target-lesion

revascularization (3.0% and 2.5%, respectively, P=0.50) and with a non-significant increase in device thrombosis for BRS (1.5% vs 0.7%, P=0.13). Similarly, the AIDA trial, which compared Absorb BVS with Xience EES with less strict inclusion criteria (enrolling patients with one or more target lesions that were considered suitable for drug-eluting stent implantation, excluding lesions more than 70 mm in length, reference vessel diameter <2.5 mm or >4 mm, bifurcation lesions intended to be treated with stenting of both main and side branch and in-stent restenosis), reported similar results: no significant difference in target-vessel failure (2-year follow-up, 11.7% for the Absorb group vs. 10.7% for the metallic EES group, P=0.43) but a significant, and even more pronounced, excess of definite and probable device thrombosis with the Absorb BVS (3.5% vs. 0.9% respectively, P<0.001). Recently, the 3-year follow-up of the ABSORB III trail have been reported. Target-vessel myocardial infarction was significantly increased in the Absorb BVS (8.6% vs 5.9%, P=0.03), as well as scaffold thrombosis (2.3% vs 0.7%, P=0.01) and ischemia-driven target-vessel revascularization (11.6% vs. 7.7%,

P=0.01).(86) Concerning scaffold thrombosis, a reference vessel diameter <2.25 mm was a predictor for scaffold thrombosis after BVS implantation; however, a significant excess of scaffold thrombosis, particularly after 12 months, was observed also in patients with a reference vessel diameter >2.25 mm (1.7% vs. 0.5%). The ABSORB IV trial compared the Absorb BVS with the Xience EES and was designed to assess if a more careful implantation technique (aggressive pre-dilation with 1:1 sized balloon, routine high-pressure post-dilation) and the avoidance of small vessels might improve the outcome of bioresorbable devices. Patients with greater complexity and at higher risk than ABSORB III were enrolled, with the inclusion of troponin-positive acute coronary syndrome and up to three lesions in a maximum of two epicardial coronary arteries, including thrombus.(79) The primary endpoint of 30-day target-lesion failure was 5.0% in the Absorb BVS group versus 3.7% in the Xience EES group, meeting thus criteria for non-inferiority (P=0.02), but a trend for increased risk of device thrombosis was still present (0.6% vs. 0.2%, P=0.06).(79)

A study-level meta-analysis of six of these trials showed that Absorb BVS compared with EES had a similar 12-month risk of target-lesion failure, target-lesion revascularization, myocardial infarction, and death.(87) However, patients treated with Absorb BVS had a higher risk of definite or probable scaffold thrombosis than those treated with metallic stents, with the highest risk observed within 30 days after implantation. Consistently, in a patient-level meta-analysis of ABSORB II, ABSORB Japan, ABOSORB China, and ABSORB III trials, patients receiving Absorb BVS showed a similar 1-year risk of patient-oriented and device-oriented clinical endpoints compared with EES, but a higher risk of target-vessel myocardial infarction, due in part to a numerically higher rate of peri-procedural events.(88) Similar results have been reported in a meta-analysis of five trials (ABSORB II, ABSORB Japan, ABSORB China, TROFI II and EVERBIO II). Patients randomly allocated to Absorb BVS incurred in a higher risk of definite/probable device thrombosis compared with Xience EES (OR 2.93, 95%CI 1.37–6.26, P=0.01), with 13 very late events occurring in 1.4% of BVS-patients, 92% of the very late

scaffold thromboses in the BVS group occurred in the absence of dual antiplatelet therapy.(89) Finally, an individual-patient data pooled meta-analysis of 4 randomized ABSORB trials (ABSORB CHINA, ABSORB II, ABSORB III, ABSORB JAPAN) was performed to assess the performance of Absorb BVS at 3-year follow-up. A total of 3,389 patients was included in the analysis. At 3-year follow-up, the use of Absorb BVS compared with Xience EES was associated with significantly higher rates of target-lesion failure (11.7% vs. 8.1%, P=0.006), driven by target vessel myocardial infarction (7.8% vs. 4.2%, P=0.0006), and ischemia-driven target-lesion revascularization (6.6% vs. 4.4%, P=0.02), and higher rates of device thrombosis (2.4% vs. 0.6%, P=0.001).(90) Between 1 and 3 years, the rate of target-lesion failure (6.1% vs. 3.9%, P=0.02) and device thrombosis (1.1% vs. 0%, P<0.0001) were higher with the Absorb BVS than Xience EES.(90)

Conclusions and perspectives

The field of interventional cardiology has witnessed unprecedented technological improvements over the past twenty years. The preferential use of the radial route,(91) the

continuous refinements in DES with the transition from early to new-generation devices,(1,3,92) along with advances in peri-procedural pharmacological therapy resulted in a substantial improvement in clinical outcomes. Considering that new-generation metallic DES have excellent efficacy and safety profile, it is challenging for newer device iterations to further improve clinical outcomes. BP-DES proved, in most cases, to have a similar safety and efficacy profile as new-generation durable polymer DES. Polymer-free DES are a promising new technology, but require further testing against new-generation DES. Emerging data seem to demonstrate that faster healing of new DES may allow shorter DAPT time, which can be of critical importance for a large, increasing number of patients (for example, frail patients at high bleeding risk or who need emergent non-cardiac surgery).(93)

Despite initial enthusiasm for the Absorb BVS, leading to test its use in more challenging scenarios, such as chronic total occlusion(94) or in-stent restenosis(95), BRS should not be preferred to conventional new-generation DES in clinical practice. The causes of relevant thrombogenicity of BVS are

still matter of debate. It has been speculated that both the resorption process and a suboptimal implant technique play a role in the occurrence of late thrombotic events. At long-term, polymeric struts are replaced by proteoglycan that is potentially thrombogenic, particularly in poorly embedded struts. Moreover, strut discontinuity may result in intraluminal dismantling, which is the prolapse of a scaffold segments into the vessel lumen as potential trigger of thrombosis.(69) However, in the next few years new iterations in polymeric BRS are expected and clinical data on metallic BRS will be available. This information will be instrumental in understanding whether bioresorbable scaffold technology may represent the standard of care in future percutaneous coronary interventions.

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Figures Legend

Figure 1. **Structure and composition of new-generation biodegradable polymer drug-eluting stents**.

BES: biolimus-eluting stent. EES: everolimus-eluting stent. NES: novolimus-eluting stent. SES=sirolimus-eluting stent. ZES=zotarolimus-eluting stent. EES=everolimus-eluting stent. Adapted with permission from Piccolo et al.(4)

Figure 4. **Structure and composition of drug-eluting bioresorbable vascular scaffolds with CE mark approval**. Abbreviations as in Figure 2. Adapted with permission from Piccolo et al.(4)

Figure 1

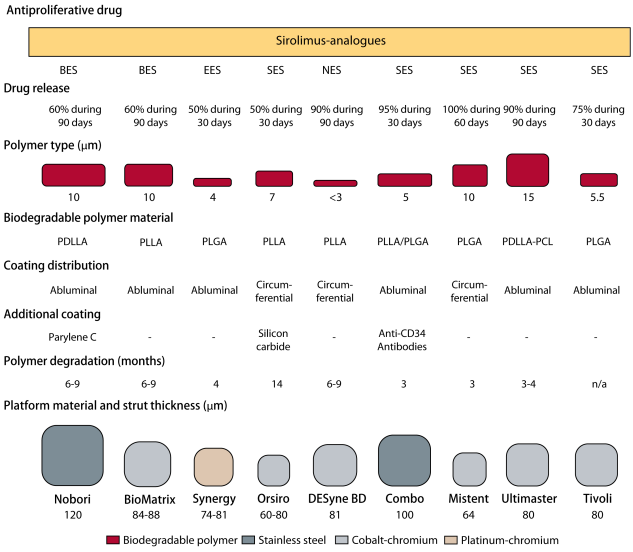
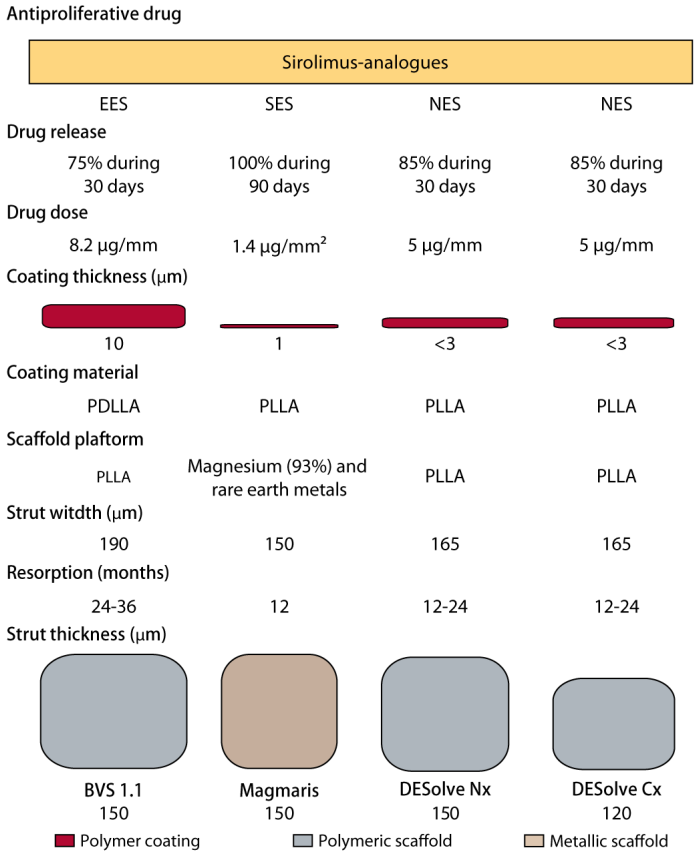

Antiproliferative drug
Sirolimus-analogues
BES
BES
EES
SES
NES
SES
SES
SES
SES
Drug release
60% during 90 days
60% during 90 days
50% during 30 days
50% during 30 days
90% during 90 days
95% during 30 days
100% during 60 days
90% during 90 days
75% during 30 days
Polymer type (μm)
10
10
4
7
<3
5
10
15
5.5
Biodegradable polymer material
PDLLA
PLLA
PLGA
PLLA
PLLA
PLLA/PLGA
PLGA
PDLLA-PCL
PLGA
Coating distribution
Abluminal
Abluminal
Abluminal
Circum-ferential
Circum-ferential
Abluminal
Circum-ferential
Abluminal
Abluminal
Additional coating
Parylene C
-
-
Silicon carbide
-
Anti-CD34 Antibodies
-
-
-
Polymer degradation (months)
6-9
6-9
4
14
6-9
3
3
3-4
n/a
Platform material and strut thickness (μm)
Nobori 120
BioMatrix 84-88
Synergy 74-81
Orsiro 60-80
DESyne BD 81
Combo 100
Mistent 64
Ultimaster 80
Tivoli 80
Biodegradable polymer
Stainless steel
Cobalt-chromium
Platinum-chromium

Figure 2


Antiproliferative drug
Sirolimus-analogues
EES
SES
NES
NES
Drug release
75% during 30 days
100% during 90 days
85% during 30 days
85% during 30 days
Drug dose
8.2 µg/mm
1.4 µg/mm²
5 µg/mm
5 µg/mm
Coating thickness (µm)
10
1
<3
<3
Coating material
PDLLA
PLLA
PLLA
PLLA
Scaffold plaftorm
PLLA
Magnesium (93%) and rare earth metals
PLLA
PLLA
Strut witdth (µm)
190
150
165
165
Resorption (months)
24-36
12
12-24
12-24
Strut thickness (µm)
BVS 1.1
150
Magmaris
150
DESolve Nx
150
DESolve Cx
120
Polymer coating
Polymeric scaffold
Metallic scaffold

Functionally Complete Coronary Revascularisation in Patients Presenting with ST-elevation MI and Multivessel Coronary Artery Disease

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Abstract

Up to half of patients undergoing primary percutaneous coronary intervention of a culprit stenosis in the context of the ST-elevation MI may present with multivessel disease. The presence of non-culprit stenoses have been shown to affect the outcomes of these patients, and the results of the more recent randomised trials highlight the importance of complete coronary revascularisation. In this paper, the authors review the main trials published on the topic and discuss tools for the assessment of non-culprit stenoses, while considering the right time for carrying out a complete coronary revascularisation.

Keywords

ST-elevation MI, non-culprit stenosis, complete revascularisation, fractional flow reserve, quantitative flow ratio

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Multivessel coronary artery disease (MVD) occurs in about half of patients presenting with ST-elevation MI (STEMI) and undergoing primary percutaneous coronary intervention (PCI), and this affects both in-hospital and long-term clinical outcomes.[1,2]

While treating the infarct-related artery (IRA) is obviously recommended, current evidence supports revascularisation of residual significant non-culprit coronary artery lesions (NCLs). However, the ideal tool for the assessment of such residual stenoses, as well as the best time for their revascularisation remain controversial, so incomplete coronary revascularisation after primary PCI continues.

In this review, we discuss the current evidence about the benefits of complete revascularisation (CR) in patients presenting with STEMI and MVD, and examine tools for the assessment of NCLs.

Complete Coronary Revascularisation versus Infarct-related Artery-only PCI

Recent findings suggest that CR in patients presenting with STEMI and MVD is associated with a better clinical outcome than primary PCI of the IRA only, regardless of whether CR was carried out during the index procedure, the index hospitalisation or during a later readmission (*Table 1*).[3–7]

In a small randomised clinical study of 214 patients presenting with STEMI, Politi et al. divided subjects into three groups: PCI of the IRA only (culprit-only revascularisation, n=84); staged revascularisation of the NCLs (n=65); and simultaneous treatment of NCLs (CR, n=65). Residual stenoses were considered angiographically significant if the diameter of the stenosis (DS) was visually estimated to be >70%.[8] At the follow-up (2.5 ± 1.4 years), 70 (32.7%) patients had experienced at least one major cardiac adverse event (MACE): 42 (50.0%) in the culprit-only revascularisation group; 13 (20.0%) in the staged revascularisation group; and 15 (23.1%) in the CR group (p<0.001).[8] The incidence of in-hospital death, repeat revascularisation and rehospitalisation was significantly higher in the culprit-only revascularisation group, whereas there was no significant difference in terms of reinfarction between the three groups, suggesting that culprit vessel-only PCI is associated with a higher rate of clinical events than multivessel treatment.[8]

In the PRAMI trial, 465 patients presenting with STEMI undergoing primary PCI of the IRA were randomised to either preventive PCI (234 patients) or no preventive PCI (231 patients).[3] Patients were eligible after primary PCI if the IRA had been successfully treated and there was a residual stenosis visually estimated to be ≥50% in at least one coronary artery other than the IRA. Recruitment was stopped early after a recommendation from the data and safety monitoring committee based on a highly significant between-group difference (p<0.001) in terms of incidence of the primary endpoint favouring preventive PCI. At a median of 23 months follow-up, the primary outcome, a composite of cardiac death, non-fatal MI or recurrent angina, occurred in 21 patients (9%) in the preventive PCI group and 53 (23%) in the group receiving PCI of the IRA only.[3]

In the DANAMI-3-PRIMULTI trial, performed at two Danish centres, 627 STEMI patients were randomised to no further invasive treatment after primary PCI of the IRA (n=313) or fractional flow reserve (FFR)-guided complete revascularisation (n=314).[5] In this study, coronary lesions with a visually estimated DS >90% were considered angiographically significant, while stenoses of 50–90% underwent FFR assessment. At a median

Table 1: Randomised Controlled Trials Comparing Complete Versus Culprit-only Percutaneous Coronary Intervention in ST-elevation MI Patients

Study	Intervention	Control	Definition of Significant Stenosis	Primary Outcome	Results
Politi et al.[8]	PCI of NCLs during either the index (CR; n=65) or a staged procedure (SR; n=65)	PCI of the culprit lesion only (COR; n=84)	Visual estimation Angio-guided NCL PCI: DS >70%	Composite of cardiac death non-cardiac death, in-hospital death, reinfarction, rehospitalisation ACS and new revascularisation	Primary outcome CR: 23% versus SR: 13% versus COR: 50% (Kaplan-Meier analysis p=0.012)
PRAMI[3]	PCI of NCLs during the index procedure (CR; n=234)	PCI of the culprit lesion only (CL; n=231)	Visual estimation Angio-guided NCL PCI: DS >50%	Composite of cardiac death, non-fatal MI and refractory angina	Primary outcome CR: 9% versus CL: 23% (HR 0.35; 95% CI [0.21–0.58])
CvLPRIT[4]	PCI of NCLs during the index procedure or index admission (CR; n=138)	PCI of the culprit lesion only (CL; n=139)	Visual estimation Angio-guided NCL PCI: DS >70% in one view or DS >50% in two views	Composite of all-cause death, recurrent MI, heart failure and ischaemia-driven revascularisation	Primary outcome CR: 10.0% versus CL: 21.2% (HR 0.45; 95% CI: [0.24–0.84])
DANAMI-3-PRIMULTI[5]	PCI of NCLs during the index admission (CR; n=314)	PCI of the culprit lesion only (CL, n=313)	Visual estimation Angio-guided NCL PCI: DS >90% FFR-guided NCL PCI: DS >50% and FFR ≤0.80	Composite of all-cause death, reinfarction and ischaemia-driven revascularisation	Primary outcome CR: 13% versus CL: 22% (HR: 0.56; 95% CI [0.38–0.83]
COMPARE ACUTE[9]	PCI of NCLs during the index procedure or index admission (CR; n=295)	PCI of the culprit lesion only (CL; n=590)	Quantitative coronary angiography FFR-guided PCI: DS >50% and FFR ≤0.80	Composite of all-cause death, non-fatal MI, any revascularisation and cerebrovascular events	Primary outcome CR: 7.8% versus CL: 20.5% (HR: 0.35; 95% CI: 0.22–0.55
COMPLETE[7]	PCI of NCLs during the index admission or staged (CR; n=2,016)	PCI of the culprit lesion only (CL; n=2025)	Visual estimation Angio-guided PCI: DS >70% FFR-guided PCI: DS 50%–69% and FFR ≤0.80	Composite of cardiovascular death and MI; and composite of cardiovascular death, MI and ischaemia-driven revascularisation	Primary outcome 1 CR: 7.8% versus CL: 10.5% HR 0.74; 95% CI: 0.60–0.91 Primary outcome 2 CR: 8.9% versus CL: 16.7% (HR 0.51; 95% CI [0.43–0.61])

ACS = acute coronary syndrome; CL = culprit lesion; COR = culprit-only revascularisation; CR = complete revascularisation; DS = diameter of stenosis; FFR = fractional flow reserve; NCL= non-culprit lesion; PCI = percutaneous coronary intervention; SR = staged revascularisation.

follow-up of 27 months, the primary endpoint (a composite of all-cause mortality, reinfarction or ischaemia-driven revascularisation of NCLs) was met in 68 (22%) patients undergoing PCI of the culprit lesion only and in 40 (13%) patients assigned to complete coronary revascularisation (HR 0.56; 95% CI [0.38–0.83]; p=0.004).[5] Of note, CR resulted in a 69% risk reduction for repeat revascularisations. No significant difference in cardiac death between the two groups was observed, but the need for both urgent and non-urgent PCI of NCLs was significantly lower in the complete revascularisation group.[5]

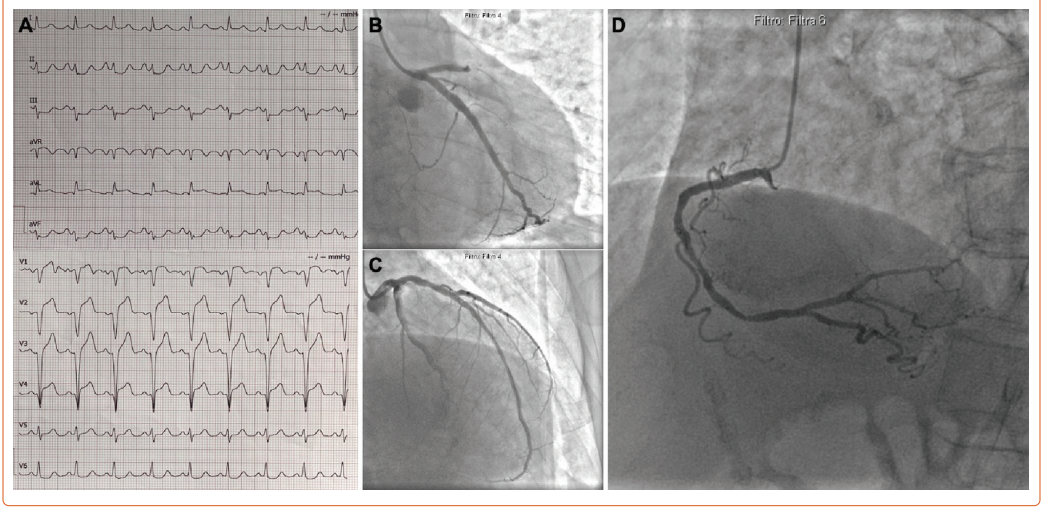
In the CvLPRIT trial, 296 patients in seven UK centres were randomised to either in-hospital complete revascularisation (n=150) or IRA-only revascularisation (n=146), with the relevance of residual coronary stenoses assessed by angiographic evaluation.[4] CR was performed either at the time of primary PCI or before hospital discharge.[4] Residual stenoses were considered angiographically significant if the DS was estimated visually to be >70% (in one view) or >50% (in two views). At 1-year follow up, MACE was significantly lower in the CR group (10.0%) than in the IRA-only group (21.2%; HR: 0.45; 95% CI [0.24–0.84]; p=0.009).[4] Cardiovascular mortality was also numerically lower in the CR group. Moreover, a trend towards a lower MACE rate was also found in patients undergoing CR during the index procedure than in those having a staged procedure.[4]

In the COMPARE-ACUTE trial, 885 patients presenting with STEMI and MVD who underwent primary PCI were assigned, at a 1:2 ratio, to FFR-guided CR (n=295) or to medical therapy (n=590).[9] Residual stenoses were considered angiographically suitable for FFR assessment if their DS was >50% by a visual estimation or quantitative angiography. The primary outcome occurred in 23 patients in the CR group and in 121 patients in the IRA-only PCI (HR 0.35; 95% CI [0.22–0.55]; p<0.001).[9] This difference was driven mainly by a significant reduction in risk of needing new revascularisations.

More recently, the COMPLETE trial showed that, among patients with STEMI and MVD, CR was superior to IRA-only PCI in reducing the risk of cardiovascular hard endpoints at a median follow-up of 3 years.[7] Residual stenoses were considered significant if, by visual estimation, DS was >70% or FFR was ≤0.80 in cases of stenoses of 50–69%. Furthermore, in this study, the investigators had to specify if they intended to perform PCI of non-culprit stenosis, either during the index procedure or 45 days later, should the patient be allocated to complete revascularisation; this allowed the investigators to evaluate whether the treatment effect of complete revascularisation versus culprit-lesion only PCI differed depending on the intended timing of non-culprit PCI.

In patients who were intended to undergo PCI during the index hospitalisation, the incidence of the two primary outcomes (cardiovascular death or MI; or cardiovascular death, MI or ischaemia-driven revascularisation) were 2.7% and 3.0% per year, respectively, in patients randomised to complete revascularisation, compared to 3.5% and 6.6% per year in those undergoing culprit-lesion-only PCI.[7] The p-values for interaction for the effect of timing on the two outcomes were p=0.62 and p=0.27, respectively, suggesting a benefit of CR regardless of whether non-culprit PCI was performed during the index hospitalisation or within 45 days after randomisation. The authors explain this benefit was the result of well-treated patients with evidence-based therapies, including

Figure 1: Stenosis After Percutaneous Coronary Intervention



Patient presenting with ST-elevation-MI and multivessel disease. A: ECG of a patient presenting with typical chest pain in the emergency room, showing the ST-segment elevation in the anterior leads and Q waves in V1–V4. Within 45 minutes, the patient was transferred to the catheterisation laboratory and underwent coronary angiography (B), which showed the total acute occlusion of the left anterior descending artery; this was rapidly treated with stent implantation and the flow was completely restored (C). However, after percutaneous coronary intervention, angiography of the right coronary artery showed an intermediate stenosis at both mid and distal segments (D).

dual antiplatelet therapy with aspirin and a $P2Y_{12}$ inhibitor, with the latter being either ticagrelor or prasugrel in the vast majority of the patients. This might have protected against early thrombotic events related to non-culprit stenosis before staged PCI.

Finally, three meta-analyses, mainly based on the cited trials, found CR was associated with a lower risk of repeat revascularisation, non-fatal MI and cardiovascular mortality compared to culprit-only PCI.[10–12]

Accordingly, both the European and the American guidelines now recommend PCI of NCLs should be considered in patients with STEMI and MVD before hospital discharge, either at the time of the primary PCI or in a staged procedure.[13–15] However, the optimal strategy for the assessment of NCLs as well as the best timing for obtaining complete revascularisation are still matter of discussion.

Invasive Evaluation of Non-culprit Lesions

In patients presenting with STEMI when NCLs are present (*Figure 1*), different clinical strategies can be considered. After the treatment of the IRA, one option is initial optimal medical therapy, with further revascularisation driven by symptom recurrence. Alternatively, the decision about the need for NCL revascularisation may be based on either angiographic or functional lesion assessment (*Figure 2*). In all cases, NCLs should be assessed during the index procedure or within a few days of the index hospitalisation, as current guidelines suggest.[13–15]

Pitfalls of Angiographic Assessment of Non-culprit Lesions

During the acute phase, severity of NCLs may be overestimated by approximately 10%, especially if visual evaluation is performed by operators with low FFR experience.[16–18] Consequently, angiography-guided NCL PCI in the acute setting might lead to functionally non-significant stenoses being treated.

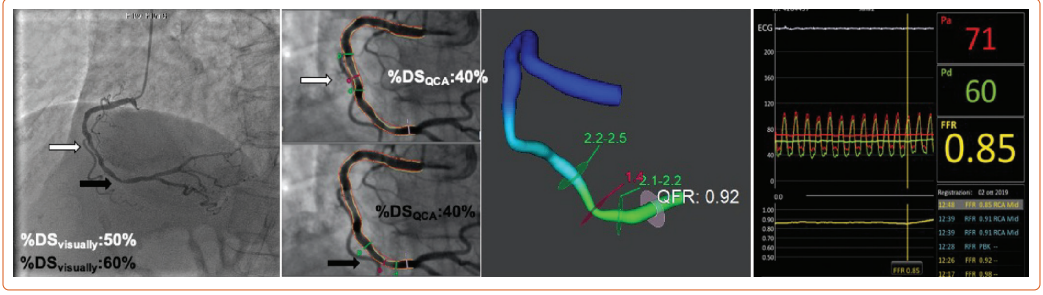
While the PRAMI and the CvLPRIT trials were based on angiographic definition of the significant residual stenosis (DS >50%), and the DANAMI-3-PRIMULTI and COMPARE ACUTE trials were based on haemodynamic assessment of residual coronary artery stenosis at the FFR evaluation (*Table 1*).[3–5,9] However, in the COMPLETE trial, while residual stenoses with a DS >70% were considered angiographically significant, those ones with a DS of 50–70% (<1%) were functionally assessed with the FFR.

Of note, when angiographic NCL evaluation was supplemented with FFR, 31% of patients randomised to CR in the DANAMI-3-PRIMULTI and 44% in the COMPARE-ACUTE trial did not need further PCI.[5,9] This demonstrates angiographic evaluation of NCLs overestimates their ischaemic potential. It also showed that if the functional assessment of NCLs is postponed until a few days after the acute setting, the risk of performing a useless invasive procedure because a negative FFR value is found ranges between 30% and 50% of cases. Furthermore, functionally non-significant NCLs may have been treated in the angiography-guided PRAMI, CvLPRIT and COMPLETE trials, suggesting that physiology would have led to the same benefit of CR although with less PCI.[3,4,7]

This is in line with the evidence that in patients with stable angina undergoing FFR-guided PCI, the residual angiographic SYNTAX score (rSS) was not predictive of adverse clinical outcome; this suggests that the functional significance of a coronary lesion is definitively the most important feature for predicting future adverse cardiac events, more so than angiographic severity, and supporting the concept of functionally complete coronary revascularisation.[19]

Because of the frequent mismatch between the angiographic and haemodynamic severities of coronary stenoses, invasive functional evaluation, even in the setting of ACS, should be considered for all residual lesions with a DS of 50–90%, as recommended by the European guidelines for the diagnosis and management of chronic coronary syndromes.[20,21]

Figure 2: Angiographic and Functional Evaluation of a Right Coronary Artery Stenosis



In the acute setting, after successful primary percutaneous coronary intervention of the left anterior descending artery, two intermediate and serial stenoses of the right coronary artery at the mid (white arrow) and distal (black arrow) segment were assessed. At the initial visual estimation, the diameter of both stenoses was >50%, while at quantitative coronary angiography assessment, they were <50%. According to the PRAMI study, the visual assessment would have been enough to support percutaneous revascularisation of the right coronary artery.[3] However, bearing in mind that angiographic assessment may result in ischaemic potential being overestimated, particularly in the acute phase of a ST-elevation MI, and to reduce the risk of performing unnecessary percutaneous coronary intervention, the residual stenosis was also assessed with QFR; the lesion was found to be functionally non-significant (QFR: 0.92) so suitable for medical therapy. This was confirmed by a FFR assessment, performed during the same procedure, which found a negative value (FFR=0.85). DS = diameter of stenosis; FFR = fractional flow reserve; Pa = coronary pressure; Pd = distal coronary pressure; QCA = quantitative coronary angiography; QFR = quantitative flow ratio.

Functional Assessment of Non-culprit Lesions Using Pressure Wire

FFR is the gold standard for the invasive assessment of the ischaemic potential of a coronary artery stenosis. It is defined as the ratio between the mean distal coronary pressure and the mean aortic pressure during maximal hyperaemia and it has been shown to be useful in several clinical and anatomic contexts.[22–26] Recently, a number of non-hyperaemic indices have been introduced and, as these are favoured because of a high correlation with the FFR but have the disadvantage of the need to induce maximal hyperaemia, they are more often used in the catheterisation laboratory.[27,28]

In the context of STEMI, functional assessment of the culprit vessel is not indicated because the dynamic changes of microcirculatory dysfunction are assessed by the index of microcirculatory resistance, leading to possible underestimation of the ischaemic potential of residual stenosis.[29,30] However, a large amount of data exists for the use of such tools for the assessment of NCLs, even though most of the current evidence supporting their use has been derived from patients presenting with chronic coronary syndrome.

However, concerns have arisen in the past about the use of FFR for the assessment of NCLs in the context of ACS, particularly in the very acute phase. In fact, it has been thought that transient coronary microcirculation dysfunction might be detected even in myocardial territories supplied by non-culprit arteries, probably due to increased neurohumoral activation and/or extravascular compression secondary to myocardial oedema.[31–35] In this clinical setting, temporary impairment of the microcirculation subtended by an equivocal stenosis would lead to the ischaemic potential of the coronary lesion being underestimated.

In another study, van der Hoeven et al. showed that FFR values measured for the assessment of NCLs during the index procedure were significantly higher than those measured at 30 days' follow-up, with a mean decrease of 0.03 units; this was particularly so in patients with larger infarcts, suggesting that the ischaemic potential of NCLs might be underestimated if FFR is used during the acute setting.[36]

However, Ntalianis et al. found there was no significant difference between FFR values measured for the assessment of NCLs during the index procedure and after one month in patients presenting with ACS.[35] Similarly, in an elegant Yorkshire pig model, Lee et al. showed that local microvascular damage induced by selective intracoronary injection of microspheres increased both the FFR and the index of microcirculatory resistance, while both remained stable in the other vessels.[37] In the Wave study, Musto and colleagues showed both the FFR and instantaneous wave-free ratio (iFR) values of NCLs did not significantly change between the index and staged procedure.[38] Finally, Mejía-Rentería et al. also support the use of FFR to assess NCLs during the subacute phase of MI; they also observed that, unlike the hyperaemic flow, which is preserved during the subacute phase of MI, the resting coronary flow is increased, which may have implications for the use of non-hyperaemic indices for the assessment of NCLs.[39] In the iSTEMI study, the iFR value of NCLs increased by a median of 0.01 from the index procedure when re-evaluation was performed within 16 days but rose by a median of 0.03 when re-evaluation was performed >16 days after primary PCI of the culprit stenosis.[40]

Taken together, these studies suggest that deferring NCL revascularisation based on both the FFR and the iFR is possible even during the acute phase of a STEMI. It should be borne in mind that the ischaemic potential of residual stenosis might be overestimated when assessed by non-hyperaemic indexes.

While the clinical relevance of acute iFR-guided PCI of NCLs is being evaluated in the iMODERN trial (NCT03298659), the benefits of deferring PCI for NCLs based on the FFR measurement have already been demonstrated in previously discussed large, randomised trials.[5–9] In addition, in a recent sub-analysis of three trials (FAME, FAMOUS-NSTEMI and DANAMI-3-PRIMULTI), a total of 547 patients presenting with ACS (271 patients with non-ST-elevation MI and 276 patients with STEMI) underwent FFR-guided functionally complete coronary revascularisation.[41] Patients with and without MACE at 2-year follow-up had a similar rSS after PCI (rSS 7.2 ± 5.5 versus 6.6 ± 5.9; p=0.23), and a Kaplan-Meier curve analysis showed a similar incidence of MACE regardless of rSS subgroup (p=0.54).[41] Therefore, even in the context of ACS, the extent of residual angiographically significant disease is not a predictor of clinical events.

Particular attention should be paid to this when with caring for elderly patients presenting with STEMI and MVD, since the benefits of complete

revascularisation, whether guided by the FFR or not, remain a matter of discussion.

A recent sub-analysis from the DANAMI-3-PRIMULTI trial showed no significant differences in the incidence of the primary endpoint in elderly patients (aged ≥75 years) randomised to culprit-only or FFR-guided complete revascularisation.[42] However, in the main study, fewer than 20% of patients were aged ≥75 years, so the question of whether FFR-guided complete revascularisation is effective also in this group has still to be answered. In the FIRE trial, the investigators aim to provide robust evidence on whether a specific revascularisation strategy should be applied to elderly patients presenting with MI and MVD to improve their clinical outcomes.[43]

Wire-free Functional Evaluation

Quantitative Flow Ratio

Quantitative flow ratio (QFR) is a novel angiography-based tool for the functional assessment of coronary artery stenoses. It has been shown to correlate with FFR, and was validated in the FAVOR and FAVOR II studies.[44,45] It is based on 3D vessel reconstructions derived from angiography and the contrast flow velocity estimated by the frame count.

Two small studies assessed the predictive value of the QFR compared with FFR to identify functionally significant NCLs. In a sub-analysis of the iSTEMI study, acute QFR showed a good diagnostic performance with both staged QFR and staged FFR as references, and a moderate diagnostic performance with staged iFR as the reference.[46]

Spitaleri et al. published a proof-of-concept study about the application of QFR for the assessment of NCLs in patients presenting with STEMI.[47] They showed an agreement between the QFR values assessed during the index (acute) and staged (3–4 days later) procedures. In addition, in a different cohort of patients, the authors found a good correlation between the FFR and QFR values of NCLs. Finally, in another cohort of patients, they showed that those with a NCL presenting with a QFR value ≤0.80 were at a higher risk of patient-oriented cardiac events (HR 2.3; 95% CI [1.2–4.5]; p=0.01).

Similarly, in the QIMERA study, QFR reassessment during a staged procedure reduced the number of significant NCLs as assessed by angiography and showed good agreement with FFR and, all patients with a QFR-negative (QFR values ≥0.82) stenosis during the index procedure remained nonsignificant at a staged assessment.[48] However, 3D-QFR requires training, might be time consuming and, at least in part, is operator-dependent, which means that the assessment may be different depending on the operator who analyses the vessel (because of his experience and skills).

Other Angiographic Scores

Recently, two angiographic tools, the Angiography-DeriveD hEmoDynamic index (ADDED index) and the DILEMMA score, have been shown to predict the FFR value.

The ADDED index is defined as the ratio between the Duke Jeopardy score, which accounts for the myocardium subtended by the coronary artery stenosis and the minimal lumen diameter acquired by quantitative coronary analysis.[49] With a cut-off value of 2.23, the ADDED index shows good diagnostic performance for predicting a positive or negative FFR value, with overall accuracy, sensitivity and specificity of 86%, 94% and 82%, respectively.[49] In patients presenting with STEMI and MVD, it has recently been shown that deferring treatment of residual stenosis on the basis of the ADDED index, rather than the visually estimated DS, is associated with a favourable clinical outcome.[50]

Similarly, the DILEMMA score takes into account the minimal lumen diameter, the lesion length and BARI (Bypass Angioplasty Revascularisation Investigation) Myocardial Jeopardy Index, and it was found to have a good correlation with FFR and a discrete accuracy in predicting significant FFR values.[51]

Such scores could help operators to discriminate functionally significant residual coronary stenosis in patients presenting with STEMI and MVD or be used to identify patients who can be safely discharged home, avoiding useless PCI or adjunctive invasive or non-invasive procedures to assess the ischaemic potential of non-culprit coronary stenosis. However, such roles have not been investigated yet.

Intracoronary Imaging to Guide Revascularisation of Non-culprit Lesions

Intracoronary imaging has also been proposed as tool for detecting significant stenosis, namely those with the features of high-risk vulnerable plaques and significantly associated with the occurrence of acute coronary syndromes (*Table 2*).[52]

Intravascular ultrasound (IVUS) is a well-established imaging technique used in the assessment of coronary plaque features. Beyond the possibility to estimate both vessel size and plaque burden, virtual histology IVUS allows operators to identify thin-cap fibroatheroma (TCFA), fibrotic plaque and fibrocalcific plaque. In the PROSPECT study, the presence of a plaque burden of ≥70%, a TCFA and a minimal lumen area ≤4 mm^2 have been suggested as independent predictors of MACE related to NCLs in patients presenting with ACS.[53]

Near-infrared spectroscopy has been also proposed to detect lipid-rich plaques. In the Lipid-Rich Plaque study, the risk of non-culprit-related MACE at two years increased of 18% for each 100-unit increase in maximum lipid core burden index.[54]

Optical coherence tomography (OCT) is a light-based imaging technique and is currently the most reliable imaging modality for TCFA detection. In the Massachusetts General Hospital OCT registry, which includes more than 1,400 patients (40% ACS), the presence of a non-culprit, lipid-rich plaque was independently associated with an increased risk of MACE related to non-culprit stenosis at 2 years.[55] In the CLIMA study of >500 patients with ACS, a minimal lumen area of <3.5mm^2, a fibrous cap thickness <75 mm, a lipid arc circumferential extension >180° and OCT-defined macrophage infiltration were all associated with an increased risk of MACE.[56]

In a OCT sub-study of the COMPLETE trial, among STEMI patients with MVD, it was found that half of patients had non-culprit lesions containing TCFA, which was more often detected in angiographic significant stenosis (DS >70% at visual estimation) than in non-obstructive lesions.[57] However, it should be underlined that both OCT and IVUS-derived indexes of plaque vulnerability have a high negative predictive value for MACE but only a low positive predictive value, which limit their clinical applicability.[53,56]

Non-invasive Assessment of Non-culprit Lesions

Before FFR was introduced, functional evaluation of intermediate coronary artery disease relied on non-invasive tests to identify the presence of stress-inducible myocardial ischaemia. Among them, exercise ECG can be considered as their forefather. Exercise ECG can be carried

Table 2: Intracoronary Imaging for the Assessment of Non-culprit Lesions

Study	n	Imaging Technique	Plaque Features
PROSPECT[53]	697 ACS	IVUS	Thin cap fibroatheroma, plaque burden ≥70%, MLA ≤4 mm^2
Lipid-Rich Plaque study[54]	1,271 (53.7% ACS)	NIRS	Lipid-rich plaque
Massachusetts General Hospital registry[55]	1,474 (39% ACS)	OCT	Lipid-rich plaque
CLIMA study[56]	1,003 (53.4% ACS)	OCT	MLA <3.5 mm^2, fibrous cap thickness <75 mm, lipid arc circumferential extension >180°, presence of macrophages

ACS = acute coronary syndrome; IVUS = intravascular ultrasound; MLA = minimum lumen area; NIRS = near-infrared spectroscopy; OCT = optical coherence tomography.

out 3–5 days after an uncomplicated ACS according to the American College of Cardiology and the American Heart Association guidelines (even though it should be submaximal).[58]

The evidence of a stress test at an acceptable cardiovascular workload (five or more metabolic equivalents) without any ECG changes, angina, hypotension, significant ST-segment depression or frequent ventricular premature contractions may show a post-MI patient is at a low risk of recurrent cardiac events; however, there are consistent limitations to using exercise ECG to assess the functional relevance of residual coronary disease. First, exercise ECG does not have spatial resolution to correctly identify myocardial ischaemia, especially in patients with MVD; in addition, changes to the ECG at rest after MI might decrease the sensibility and the predictive value of the test.

Dobutamine stress echocardiography has been proven to be safe when performed 5 days after MI.[59] Previous studies have shown that stress echocardiography might be an efficient tool to detect the presence of myocardial ischaemia, including in myocardial territories not supplied by the culprit artery in patients with MVD.[60]

Coronary flow velocity reserve (CFVR) by transthoracic Doppler echocardiographic imaging might be also useful in the assessment of non-culprit coronary artery stenosis. Tesic et al. enrolled 230 patients with residual intermediate (50%–70%) stenosis of the non-infarct-related arteries, in whom CFVR was performed within 7 days of primary PCI. The authors found that deferring patients with intermediate residual stenosis with a CFVR >2 was safe and associated with excellent long-term clinical outcomes. However, this tool is particularly affected by some limitations due to the acoustic window of the patients and the feasibility of the technique to assess flow in different coronary arteries. The evaluation of CFVR is more feasible in the left anterior descending artery and right coronary artery than the left circumflex artery.[61]

Similarly, quantitative myocardial single photon emission CT (SPECT) has been used largely to detect residual myocardial ischaemia after acute MI.[62] Stress echocardiography and myocardial SPECT are equally accurate for detecting MVD early after acute MI.[60]

However, unlike with the FFR-guided strategy, there are no studies evaluating the prognostic impact of a non-invasive, imaging-based strategy to guide myocardial revascularisation of residual non-culprit coronary artery disease in patients with STEMI.[63] The same applies to perfusion cardiac MRI.

FFR derived from computed coronary angiography (FFR-CT) deserves the final mention in this setting. Computation of the FFR from standard acquired coronary CT angiography datasets has recently been developed. The diagnostic performance of FFR-CT in identifying functional significant stenosis using the FFR as the standard of reference is high and superior to anatomical interpretation in patients with stable angina; in addition, its application for the evaluation of NCLs in patients presenting with STEMI has recently been evaluated in a prospective, single centre study where patients undergoing primary PCI with at least one equivocal stenosis in a non-culprit vessel were subjected to coronary CTA after 1 month.[64] Using computational fluid dynamics principles, coronary blood flow and pressures were computed under simulated hyperaemic conditions; lesion-specific ischaemia was defined as FFR-CT <0.80 as in previous studies. However, in this study, the overall diagnostic performance of FFR-CT for staged detection of functional significant NCLs appeared to be modest.[64]

Cardiac MRI might also be considered to evaluate patients with suspected obstructive coronary artery disease. Cardiac MRI does not expose patients to ionising radiation and allows high-resolution imaging to be obtained. It is also possible to quantify myocardial blood flow in both relative and absolute terms.[65,66] In a sub-study of the REDUCE-MVI trial, Everaars et al. found a moderate agreement between CMR and FFR for the assessment of non-culprit stenosis in patients presenting with STEMI and MVD. However, the sample size was limited and mainly underpowered for this purpose, so randomised trials would be useful for supporting this tool for the assessment of non-culprit stenosis in the setting of ACS.[67,68]

Conclusion

The correct management of residual coronary artery disease in patients with STEMI and MVD undergoing primary PCI remains a concern. Issues remain regarding the correct timing and guiding criteria for interventions. The introduction of FFR together with the concept of functionally complete coronary revascularisation is surely a critical innovation. However, functional measures, such as the FFR and/or its surrogates should not completely supplant clinical judgement.

Lesion vulnerability, patient comorbidities, size of ischaemic territory, ability to comply with dual antiplatelet therapy and risk of contrast-induced kidney injury are only some of the issues that should be considered when pursuing complete revascularisation.

Larger studies, such as FULL REVASC (NCT02862119) and FRAME-AMI (NCT02715518), will add further knowledge to this complex and interesting field. ❑

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ADDED Index or percentage diameter of residual coronary stenosis to risk-stratify patients presenting with STEMI☆

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ABSTRACT

Background: We compared the prognostic value of the ADDED Index with visually estimated diameter (DS) of residual coronary stenosis (RS) in STEMI patients after successful PCI of the culprit lesion.
Even though associated with a positive outcome, the functional assessment of non-culprit stenosis remains largely underused, especially in STEMI patients. The Angiography-DeriveD hEmoDynamic index (ADDED index) showed high accuracy to predict FFR and it might be used to better guide the diagnostic and therapeutic work-up of such patients.
Methods: We retrospectively included 596 patients grouped on the basis of either the ADDED Index (ADDED Negative (<2.23, n = 153) vs ADDED Positive (≥2.23, n = 129)) or the DS of the RS (RS Negative (<50%, n = 177) vs RS Positive (≥50%, n = 105)). Patients without any RS served as control (n = 314). Primary endpoints were: 1) major adverse cardiac events (MACE), composite of all-cause death, myocardial infarction (MI), clinically driven revascularizations (CDR); 2) non-culprit vessel oriented clinical events (VOCE), composite of all-cause death, non-culprit vessel related MI and CDR.
Results: At 24 months the rate of both MACE and VOCE was significantly higher in both the ADDED Positive and RS Positive groups. However, differently from patients in whom complete revascularization was deferred on the basis of the angiography (RS Negative), no additional risk was found for patients in the ADDED Negative group.
Conclusions: In STEMI patients with MVD deferring treatment of RS on the basis of the ADDED index, rather than the visually estimated DS, is associated with a favorable clinical outcome.

1. Introduction

Multivessel coronary artery disease (MVD) is commonly observed in patients presenting with STEMI, occurring in about half of the patients undergoing PCI with significant impact on in-hospital and long-term clinical outcomes [1,2]. While it is recommended to treat the "culprit" stenosis, evidence supporting revascularization of residual "significant" coronary artery stenosis is conflicting [3]. Recent randomized trials showed improved clinical outcomes in patients undergoing complete revascularization as compared with patients in whom culprit-only PCI was performed [4–7]. Although a FFR-guided complete coronary revascularization was associated with a significant advantage in terms of cardiovascular events, in daily practice the vast majority of decisions about revascularization are based on the visually estimated diameter stenosis (DS) assessed at the angiography, being a DS ≥50% historically used as cut-off to justify revascularization [6,8–10]. However, FFR measurement of non-culprit coronary artery stenosis remains largely underused. A new recently validated Angiography-DeriveD hEmoDynamic index (ADDED index), taking into account both the minimal lumen diameter of a coronary artery stenosis and the amount of jeopardized myocardium subtended, was able to predict the functional significance of a certain intermediate coronary artery stenosis, due to its high correlation with the FFR [11]. The aim of the present retrospective study was to compare the prognostic value of the ADDED Index with DS of residual coronary stenosis (RS) in patients presenting with STEMI and MVD, after successful PCI of the culprit lesion.

2. Materials and methods

All consecutive patients presenting with STEMI who referred to our department for primary, rescue or elective (after successful thrombolysis) PCI between January 2013 and December 2015 were included.

☆ The authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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Diagnosis of STEMI and clinical/interventional management was made according to the current guidelines [3]. PCI was performed according to the conventional strategies and operators' routine practice. Patients with previous CABG, CTO, diffusely diseased main vessels, undergoing not successful PCI of the culprit stenosis, or patients who did not survive after the acute procedure were excluded. In patients with MVD, the decision to perform non-culprit vessel PCI, either during the index procedure or in a staged session, was left to the operator choice. Staged PCI was defined as revascularization of at least one non-culprit lesion during the index hospital admission or planned in the following 30 days. Digitally archived angiograms were reviewed by two interventional cardiologists blinded to clinical outcomes. Intermediate coronary artery stenosis was defined on the basis of visual estimation as those determining a reduction of vessel diameter comprised between 30% and 75% [12]. Two-dimensional QCA was performed offline using the cardiovascular angiography analysis system (Pie Medical Imaging, Maastricht, the Netherlands) and reference vessel diameter (RVD), lesion length (LL) and minimal lumen diameter (MLD) were calculated. The amount of perfused myocardium subtended by the target stenosis was assessed using the Duke Jeopardy Score (DJS) as previously reported [11,13,14]. The ADDED index for a single vessel was defined as the ratio between the DJS and the MLD [11].

After completion of eventually staged procedures, patients were grouped as following:

a) According to the ADDED Index of the RS:
1. Patients with at least one RS with an ADDED Index value ≥ 2.23 (ADDED Positive group)
2. Patients with one or more RS with an ADDED Index value < 2.23 and no RS with an ADDED index ≥ 2.23 (ADDED Negative group)

b) According to the visually estimated DS of the RS:
1. Patients with at least one RS with a DS ≥ 50% (RS Positive group)
2. Patients with one or more RS with a DS < 50% and no RS with a DS ≥ 50% (RS Negative group)

Patients without any RS served as control (Control group).

The study conformed to the Declaration of Helsinki on human research, and all patients gave informed consent.

Demographic, clinical, echocardiographic, angiographic and laboratory data at admission were collected and recorded in a computerized database, in accordance with our department's protocol for patients with STEMI undergoing PCI. Clinical follow-up was performed using hospital records and telephone interviews.

Primary endpoints were: 1) Major Adverse Cardiac Events (MACE), defined as the composite of all-cause death, myocardial infarction (MI), clinically driven revascularization; 2) deferred non-culprit Vessel-Oriented Clinical Events (VOCE), defined as composite of all-cause death, deferred non-culprit vessel related MI and clinically driven revascularizations. Staged procedures were not considered as events. All events were classified and adjudicated by a physician who was unaware of the study group and of the angiographic details of the lesions.

Continuous variables are presented as mean ± standard deviation (SD) or median with interquartile range (IQR) as appropriate. Normal distribution was assessed by the Kolmogorov-Smirnov test. Categorical variables are reported as frequencies and percentages. Comparisons between categorical variables were evaluated using the Pearson $\chi 2$ test. Continuous variables were compared using one-way ANOVA. A propensity score was built with a non-parsimonious method to account for potential differences in treatment allocation and then entered into a logistic regression model, considering complete revascularization as the dependent variable. In particular, all variables listed in Tables 1A, 1B, 2A and 2B with a p value <0.05 were incorporated into the model, and the score was then used in the proportional hazards analyses as a covariate. The three groups have been analyzed pairwise. Clinical end points were evaluated by Kaplan-Meier method and Cox proportional hazard analysis. Data were analyzed with SPSS, version 25.0, software (SPSS Inc., Chicago, Illinois) and GraphPad Prism 7.00 (GraphPad Software Inc., La Jolla, California). A probability value of <0.05 was considered significant.

Table 1A
Clinical characteristics.

	No residual stenosis	Residual stenosis		p value
	Control group	ADDED Index Negative	ADDED Index Positive	
n	314	153	129	
Age	59 ± 12	63 ± 12	64 ± 12	<0.001
Male gender	244(78)	116(76)	100(77)	0.90
LVEF (%)	44 ± 8	44 ± 7	42 ± 8	0.12
Diabetes	58(18)	28(18)	38(29)*	0.02
Hypertension	169(54)	90(59)	89(69)	0.01
Hyperlipidaemia	112(36)	58(38)	64(50)	0.02
Smoker	131(42)	53(35)	49(38)	0.32
Previous MI	23(7)	12(8)	16(12)	0.21
Previous PCI	19(6)	12(8)	10(8)	0.70
PAD	1(0)	3(2)	6(5)	0.01
CKD	7(2)	2(1)	7(5)	0.08
Atrial fibrillation	2(1)	1(1)	4(3)	0.08

(CKD: Chronic Kidney Disease, LVEF: Left Ventricle Ejection Fraction, MI: Myocardial Infarction, PAD: Peripheral Artery Disease, PCI: Percutaneous Coronary Intervention, *: $p < 0.05$ vs ADDED Negative group).

3. Results

A total of 596 patients were included and 314 (53%) underwent complete coronary revascularization (Control group) because presenting with one single vessel disease or undergoing PCI of non-culprit stenosis (29%), mainly in a staged procedure, on the basis of the operator's choice. Patients with at least one RS were grouped either according to the ADDED Index value (153 patients in the ADDED Negative group and 129 in the ADDED Positive group) or to the DS of the RS (177 patients in the RS Negative group and 105 patients in the RS Positive group). Among these patients, the ADDED Index provided an important reclassification; in fact, as shown in the Supplementary Table 1S, 41% of patients with a RS deemed to be significant (≥50%) at the visual estimation (RS Positive) have been reclassified as Negative according to the ADDED Index (ADDED Index Negative), while 38% of patients with a non-significant RS (<50%) at the visual estimation (RS Negative) have been reclassified as Positive on the basis of the ADDED Index (ADDED Index Positive).

Table 1B
Clinical characteristics.

	No residual stenosis	Residual stenosis		p value
	Control group	RS Negative	RS Positive	
n	314	177	105	
Age	59 ± 12	63 ± 12	65 ± 13	<0.001
Male gender	244(78)	140(79)	76(72)	0.41
LVEF (%)	44 ± 8	43 ± 8	43 ± 7	0.41
Diabetes	58(18)	40(23)	26(25)	0.29
Hypertension	169(54)	110(62)	69(66)	0.04
Hyperlipidaemia	112(36)	77(43)	45(43)	0.16
Smoker	131(42)	66(37)	36(34)	0.34
Previous MI	23(7)	19(11)	9(9)	0.44
Previous PCI	19(6)	14(8)	8(8)	0.70
PAD	1(0)	5(3)	4(4)	0.02
CKD	7(2)	6(3)	3(3)	0.75
Atrial fibrillation	2(1)	3(2)	2(2)	0.43

(CKD: Chronic Kidney Disease, LVEF: Left Ventricle Ejection Fraction, MI: Myocardial Infarction, PAD: Peripheral Artery Disease, PCI: Percutaneous Coronary Intervention).

Table 2A
Angiographic and procedural characteristics.

	No residual stenosis	Residual stenosis		p value
	Control group	ADDED Index Negative	ADDED Index Positive	
n	314	153	129	
Revascularization strategy				0.51
Elective PCI	61(19)	26(17)	23(18)	
Rescue PCI	58(18)	20(13)	20(15)	
Primary PCI	195(62)	107(70)	86(67)	
Number of diseased vessels				<0.001*
1	222(71)	0(0)	0(0)	
2	82(26)	107(70)	61(47)	
3	10(3)	46(30)	68(53)	
MVD	92(29)	153(100)	129(100)	<0.001
Target vessel PCI				<0.001*
LAD	173(55)	87(57)	47(36)	
LCX	32(10)	23(15)	23(18)	
RCA	109(35)	43(28)	59(46)	
Stent diameter	3.1 ± 0.4	3.0 ± 0.4	3.0 ± 0.4	<0.001
Stent length	26 ± 14	29 ± 16	28 ± 15	0.07
Stent type				0.10
Bare metal stent	28(9)	8(5)	15(12)	
Drug eluting stent	286(91)	145(95)	114(88)	
Non-culprit PCI	92(29)	30(20)	31(24)	0.07
Mutivessel PCI	33(36)	9(30)	8(26)	0.55
Residual vessel				
LAD		39(25)	79(61)	<0.001
DS(%)		46(37–50)	45(40–56)	0.21
ADDED Index		1.54 (1.18–1.82)	3.08 (2.61–3.62)	<0.001
LCX		74(48)	68(53)	0.48
DS(%)		45(37–53)	57(49–70)	<0.001
ADDED Index		1.43 (1.20–1.67)	2.39 (1.56–3.32)	<0.001
RCA		67(44)	38(29)	<0.001
DS(%)		43(38–50)	48(40–61)	0.07
ADDED Index		1.11 (0.92–1.41)	1.54 (1.02–2.50)	0.02

(DS: Diameter Stenosis, LAD: Left Anterior Descending Artery, LCX: Left Circumflex, MLD: Minimal Lumen Diameter, PCI: Percutaneous Coronary Intervention, RCA: Right Coronary Artery, TV: Target Vessel, *: $p < 0.05$ ADDED Positive vs ADDED Negative group).

Table 2B
Angiographic and procedural characteristics.

	No residual stenosis	Residual stenosis		p value
	Control group	RS Negative	RS Positive	
n	314	177	105	
Revascularization strategy				0.34
Elective PCI	61(19)	28(16)	21(20)	
Rescue PCI	58(18)	23(13)	17(16)	
Primary PCI	195(62)	126(71)	67(64)	
Number of diseased vessels				<0.001*
1	222(71)	0(0)	0(0)	
2	82(26)	125(71)	43(41)	
3	10(3)	52(29)	62(59)	
MVD	92(29)	177(100)	105(100)	<0.001
Target vessel PCI				0.19
LAD	173(55)	85(48)	49(47)	
LCX	32(10)	28(16)	18(17)	
RCA	109(35)	64(36)	38(36)	
Stent diameter	3.1 ± 0.4	3.0 ± 0.4	3.0 ± 0.4	0.005
Stent length	26 ± 14	27 ± 13	32 ± 19	0.005
Stent type				0.35
Bare metal stent	28(9)	14(8)	9(9)	
Drug eluting stent	286(91)	163(92)	96(91)	
Non-culprit PCI	92(29)	34(19)	27(26)	0.049*
Mutivessel PCI	33(36)	10(29)	7(26)	0.56
Residual vessel				
LAD		68(38)	50(48)	0.14
DS(%)		40(36–46)	55(44–60)	<0.001
ADDED Index		2.27 (1.54–3.09)	2.86 (1.82–3.61)	0.03
LCX		72(41)	72(69)	<0.001
DS(%)		41(35–45)	59(52–70)	<0.001
ADDED Index		1.43 (1.16–1.82)	1.82 (1.43–2.62)	<0.001
RCA		64(36)	41(39)	0.70
DS(%)		40(35–47)	54(45–65)	<0.001
ADDED Index		1.05 (0.90–1.20)	1.43 (1.00–1.96)	0.01

(DS: Diameter Stenosis, LAD: Left Anterior Descending Artery, LCX: Left Circumflex, MLD: Minimal Lumen Diameter, PCI: Percutaneous Coronary Intervention, RCA: Right Coronary Artery, TV: Target Vessel, *: $p < 0.05$ RS Positive vs RS Negative group).

When the ADDED Index was used to classify patients with a RS, those included in the ADDED Positive group were significantly older, with higher prevalence of diabetes mellitus, hypertension, hyperlipidemia and peripheral artery disease (Table 1A). A similar proportion of patients among groups underwent primary PCI. Left Anterior Descending Artery (LAD) was more often the culprit vessel of patients included in both the Control and the ADDED Negative groups as compared with the ADDED Positive group (Table 2A). No significant difference was found between the three groups in terms of proportion of DES implanted for the treatment of the culprit stenosis. PCI of non-culprit stenosis was performed, at operator's discretion, in 92 (29%), 30 (20%) and 31 (24%) patients respectively included in the Control, ADDED Negative and ADDED Positive groups. Of note, among patients included in the ADDED Positive group, as compared with the ADDED Negative group, the LAD was more often left untreated despite the presence of a RS with a comparable DS at visual estimation. The Left Circumflex (LCX) was equally left untreated in half of the patients included in the two groups while the right coronary artery (RCA) was more often left untreated in the ADDED Negative group.

When visually estimated DS was used to classify patients with a RS, those included in the RS Positive group (DS ≥ 50%) were significantly older, with higher prevalence of hypertension and peripheral artery disease (Table 1B). A similar proportion of patients among groups underwent primary PCI and the LAD was more often the culprit vessel. No significant difference was found between the three groups in terms of proportion of DES implanted for the treatment of the culprit stenosis (Table 2B). Distribution of the residual vessels was similar among the two groups excepted for the LCX which was more often left untreated in the RS Positive group.

Clinical follow-up was obtained in 589 (99%) of 596 patients at a median of 24 months (14–40 month). At the Kaplan-Meier analysis, both MACE-free and VOCE-free survival were significantly lower in the ADDED Positive group as compared with both the ADDED Negative and the Control groups (p for trend <0.001). In addition, no significant difference was found between the ADDED Negative and Control groups (MACE: $p = 0.81$ and VOCE: $p = 0.42$) (Fig. 1, panels A and B). At the Propensity Score adjusted Cox's analysis, (Table 3A), MACE rate was significantly higher in the ADDED Positive group (28%) as compared with both the ADDED Negative (6%, hazard ratio [HR] 4.96, 95% confidence interval [CI] 2.38–10.32, $p < 0.001$) and the Control groups (7%, HR: 3.27 [1.54–6.93], $p < 0.001$). This difference was mainly driven by the higher rate of overall clinically driven revascularizations and the higher incidence of MI. Similarly, the rate of VOCE was significantly higher in patients included in the ADDED Positive group (25%) as compared with both the ADDED Negative (5%, HR: 5.63 [2.46–12.79], $p < 0.001$) and the Control groups (3%, HR: 10.09 [3.33–30.56], $p < 0.001$). This difference was mainly driven by the higher rate of deferred non-culprit vessel related MI and clinically driven revascularizations.

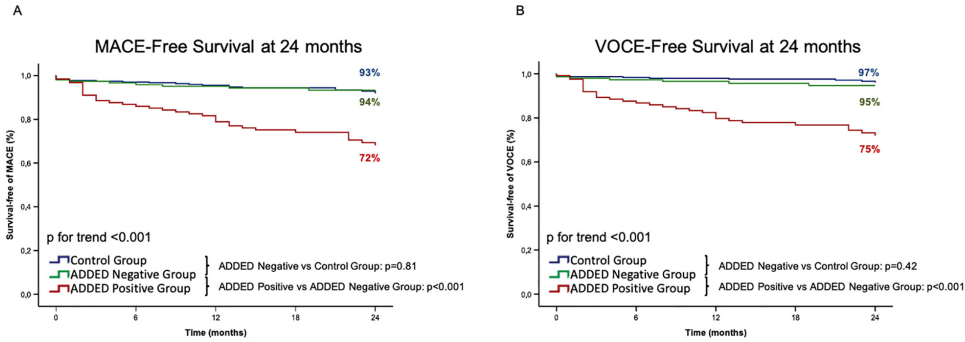

Fig. 1. Kaplan-Meier analysis of the two-primary endpoints at 2-years follow-up in patients grouped according to the ADDED Index of RS. Panel A. MACE-free survival was significantly lower in the ADDED Positive group as compared with both the ADDED Negative and the Control groups. Of note, no significant difference was found between the Control Group and the ADDED Negative group. Panel B. VOCE-free survival was significantly lower in the ADDED Positive group as compared with both the ADDED Negative and the Control groups. Of note, no significant difference was found between the Control Group and the ADDED Negative group.

When patients were classified according to the visually estimated DS of the RS (Table 3B), at the Kaplan-Meier analysis, both MACE-free and VOCE-free survival were significantly lower in the RS Positive group as compared with both the RS Negative and the Control groups (p for trend <0.001). In addition, no significant difference was found between the RS Negative and the RS Positive groups (MACE: $p = 0.13$ and VOCE: $p = 0.11$) while a significant difference was also found between the RS Negative and Control groups (MACE: $p = 0.01$ and VOCE: $p < 0.001$) (Fig. 2, panels A and B). At the Propensity Score adjusted Cox's analysis, (Table 3B), patients included in the RS Positive group showed only a trend towards higher MACE rate (19%) as compared with Control group (7%, HR: 2.24 [0.98–5.12], $p = 0.06$). In addition, the rate of MACE did not significantly differ between the RS Positive and the RS Negative groups (14%, HR: 1.53 [0.84–2.77], $p = 0.16$). Furthermore, the rate of VOCE was significantly higher in patients included in the RS Positive group (17%) as compared with Control group (3%, HR: 6.64 [2.01–21.86], $p < 0.001$). Conversely, the rate of VOCE did not significantly differ between the RS Positive and the RS Negative groups (11%, HR: 1.63 [0.86–3.10], $p = 0.13$).

4. Discussion

In the present study we showed the usefulness of the ADDED Index to identify those patients presenting with STEMI and multivessel disease who would benefit the most of complete myocardial revascularization. In fact, after PCI of the culprit stenosis, deferring treatment of RS with an ADDED Index ≥2.23 is associated with a significant higher risk of cardiovascular events. In the ADDED Positive group indeed, the higher incidence of both MACE and VOCE was mainly driven by the higher rate of both myocardial infarctions and clinically driven revascularizations. Of note, clinical outcome of patients included in the ADDED Negative group, namely those with one or more RS with a negative

Table 3A
Clinical events at 24 months follow up.

	Control group	ADDED Index Negative	PS adjusted HR (95%CI)	*p* value	ADDED Index Positive	PS adjusted HR (95%CI)	*p* value
n	311	152			126		
Overall death	10 (3)	5 (3)			7 (6)		
	Reference		2.09 (0.33–13.08)	0.43		3.14 (0.59–16.73)	0.18
		Reference				1.44 (0.45–4.63)	0.54
Non-fatal MI	8 (3)	3 (2)			10 (8)		
	Reference		0.51 (0.11–2.42)	0.40		2.19 (0.63–7.62)	0.41
		Reference				4.06 (1.12–14.76)	0.03
Deferred non-culprit vessel related non-fatal MI	–	1 (1)			6 (5)		
	Reference		–	–		–	–
		Reference				6.84 (0.82–57.30)	0.08
Overall revascularization	10 (3)	3 (2)			27 (21)		
	Reference		0.40 (0.09–1.76)	0.23		6.30 (2.07–19.19)	0.001
		Reference				11.33 (3.43–37.38)	<0.001
Deferred non-culprit vessel related revascularization	–	2 (1)			24 (19)		
	Reference		–	–		–	–
		Reference				15.14 (3.57–64.11)	<0.001
VOCE	10 (3)	7 (5)			31 (25)		
	Reference		2.19 (0.30–15.79)	0.44		10.09 (3.33–30.56)	<0.001
		Reference				5.63 (2.46–12.79)	<0.001
MACE	21 (7)	9 (6)			35 (28)		
	Reference		0.46 (0.06–3.48)	0.45		3.27 (1.54–6.93)	<0.001
		Reference				4.96 (2.38–10.32)	<0.001

Table 3B
Clinical events at 24 months follow up.

	Control group	RS < 50%	PS adjusted HR (95%CI)	*p* value	RS ≥ 50%	PS adjusted HR (95%CI)	*p* value
n	311	175			103		
Overall death	10 (3)	7 (4)			5 (5)		
	Reference		2.48 (0.43–14.28)	0.31		2.83 (0.49–16.24)	0.24
		Reference				1.01 (0.32–3.22)	0.98
Non-fatal MI	8 (3)	9 (5)			4 (4)		
	Reference		1.27 (0.38–4.26)	0.70		0.95 (0.23–3.96)	0.94
		Reference				0.81 (0.25–2.66)	0.73
Deferred non-culprit vessel related non-fatal MI	–	5 (3)			2 (2)		
	Reference		–	–		–	–
		Reference				0.76 (0.15–3.98)	0.75
Overall revascularization	10 (3)	14 (8)			16 (15)		
	Reference		1.59 (0.55–4.54)	0.39		3.64 (1.23–10.77)	0.02
		Reference				2.25 (1.09–4.63)	0.03
Deferred non-culprit vessel related revascularization	–	13 (7)			13 (13)		
	Reference		–	–		–	–
		Reference				2.01 (0.93–4.36)	0.08
VOCE	10 (3)	20 (11)			18 (17)		
	Reference		9.17 (2.72–30.86)	<0.001		6.64 (2.01–21.86)	<0.001
		Reference				1.63 (0.86–3.10)	0.13
MACE	21 (7)	24 (14)			20 (19)		
	Reference		3.08 (0.98–9.67)	0.05		2.24 (0.98–5.12)	0.06
		Reference				1.53 (0.84–2.77)	0.16

ADDED Index value, did not differ significantly as compared with the Control group. Differently, when patients were grouped on the basis of visually estimated DS, although those with a positive (DS ≥ 50%) RS showed the highest incidence of both MACE and VOCE, patients' clinical outcome did not differ significantly from patients with a negative (DS < 50%) RS, being these latter patients at higher risk of clinical events as compared with Control group, suggesting that visual estimation of RS should not be used to safely defer revascularization because of the higher risk to underestimate the ischemic potential of RS, particularly those subtending large myocardial mass.

Taken together, these results suggest that the ADDED Index might help to decide whether or not to treat intermediate coronary artery stenosis after PCI of the culprit stenosis in STEMI patients with MVD. On the contrary, a decision based on the visual estimation of the RS should not be adopted to defer a complete coronary revascularization, particularly in this clinical setting.

Routine revascularization of "significant" non-culprit stenosis should be considered in STEMI patients with MVD before hospital discharge, preferably during a staged procedure [3]. In this setting, a complete coronary revascularization was associated with a significant reduction of cardiovascular events, as compared with patients in whom the culprit lesion was only treated [3,7]. However, the decision of performing or not the PCI of the non-culprit stenosis may be challenging when facing with intermediate coronary artery stenosis. Angiographic assessment of lesion severity conveys a significant risk to overestimate or underestimate the functional significance of intermediate coronary artery stenosis, particularly those supplying respectively small and large myocardial territories [4,15]. The usefulness of FFR in the setting of the acute coronary syndromes has been well established as well as for stable coronary artery disease [6,16]. In particular, a FFR-guided complete revascularization of non-culprit stenosis, after primary PCI, showed to be safe and associated with a reduction in cardiovascular

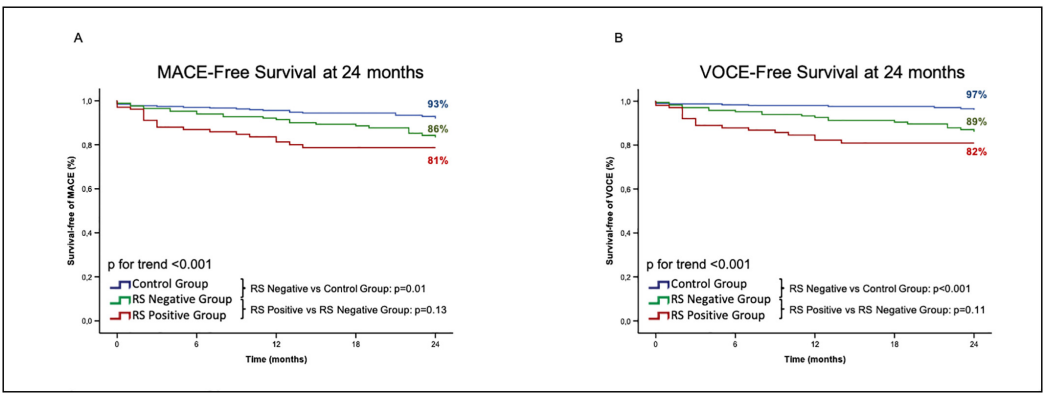

Fig. 2. Kaplan-Meier analysis of the two-primary endpoints at 2-years follow-up in patients grouped according to the visually estimated diameter of RS. Panel A. MACE-free survival was significantly lower in the RS Positive group. Of note, a significant difference was also found between the Control group and the RS Negative group, while no significant difference was found between this latter group and the RS Positive group. Panel B. VOCE-free survival was significantly lower in the RS Positive group. Of note, a significant difference was also found between the Control group and the RS Negative group, while no significant difference was found between this latter group and the RS Positive group.

hard endpoints. However, FFR is still underused particularly in ACS patients, probably because of concerns on procedural time duration, adenosine administration and pressure-wire manipulation [16–18]. Hence, planning a staged procedure to perform a PCI of an intermediate coronary artery stenosis on the basis of the visual estimation of diameter stenosis would increase the risk of performing PCI for "non-functionally" significant coronary artery stenosis, thereby increasing both the risks and costs for procedures that are not really necessary from a clinical standpoint. Moreover, in a not negligible percentage of cases, staged measurement of FFR finds not-functionally significant coronary stenosis while exposing the patient to all the potential risk of an invasive procedure. At this regard, indeed, the percentage of significant (DS ≥ 50%) non–culprit coronary artery stenosis with a negative FFR Value (>0.80) was 31% in the DANAMI-3–PRIMULTI trial and 50% in the Compare Acute trial [6,16]. To further extend the available evidence, in our patients' population, 41% of stenosis stated "significant" at visual estimation (DS ≥ 50%) had a negative ADDED Index value (<2.23), thereby with a high risk to find a negative FFR value if this latter would have been measured. On the contrary, 38% of the stenosis stated "not significant" at visual estimation (DS < 50%) had a positive ADDED Index value (≥2.23) at high risk, indeed, to be functionally significant. Therefore, to overcome these limitations, the assessment of non-culprit intermediate coronary artery stenosis is generally postponed to a later stage by performing non-invasive imaging tests, bearing in mind all the potential limitations associated with such non-invasive procedures [19].

Thereby, an angiographic-based tool able to predict the FFR value, drug- and wire-free, would limit staged procedures to functionally significant coronary artery stenosis only, thereby reducing the risk of performing clinically unnecessary coronary interventions.

The ADDED Index complies with this function, allowing to safely defer, better than the visual estimation, the treatment of non-functionally significant coronary artery stenosis without increasing the risk of major cardiovascular events at long term follow up. In addition, such index might be used to plan staged procedures for patients who would benefit the most from functionally complete coronary revascularization. Finally, the ADDED Index might decrease the need for further non-invasive testing or repeated unnecessary catheterizations and therefore shorten diagnostic work-up after an acute MI.

This study might be affected by the limitations inherent to all retrospective registries: that is, events underreporting, low event rate, especially for death, bias related to the operator's decision as to the revascularization strategy to be adopted, and many other potential confounding factors. In particular, the decision to perform a complete coronary revascularization might have been influenced by several clinical and procedural features; these limitations remain, although we tried to minimize their impact by performing a propensity score adjusted Cox regression analysis to assess the clinical outcome. FFR was not applied to guide revascularization of non-culprit intermediate coronary artery stenosis and non-invasive functional testing was available only in a few patients but we are unable to evaluate whether they have been used for PCI guidance. Even though most of the staged procedures (95%) were performed during the index hospitalization, we cannot exclude that some deferred non-culprit PCI, particularly those occurred within 6 months (n = 7) from discharge, might have been originally planned later than 30 days for several reasons that we cannot account due to the retrospective nature of the present study. Both MLD and visually estimated DS was derived by the angiography performed during the acute phase and, in such adrenergic context, this might have leaded to an overestimation of the stenosis severity.

5. Conclusions

Among patients presenting with STEMI and MVD, calculation of the ADDED Index, rather than the visually estimated diameter, of the residual coronary artery stenosis, allows to identify those patients who would benefit the most from a complete coronary revascularization while avoiding unnecessary PCI or adjunctive invasive or non-invasive procedures to assess the functional significance of non-culprit coronary stenosis.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.carrev.2021.01.030.

CRediT authorship contribution statement

Luigi Di Serafino: Conceptualization, Writing – original draft. **Fabio Magliulo:** Data curation, Formal analysis. **Emanuele Barbato:** Writing – review & editing, Resources. **Plinio Cirillo:** Writing – review & editing, Resources. **Mafalda Esposito:** Data curation, Formal analysis. **Federica Serino:** Methodology, Software, Formal analysis. **Francesca Ziviello:** Methodology, Software, Formal analysis. **Eugenio Stabile:** Writing – review & editing, Resources. **Anna Franzone:** Writing – review & editing, Validation. **Raffaele Piccolo:** Writing – review & editing, Validation. **Francesco Borgia:** Writing – review & editing, Validation. **Carmine Morisco:** Visualization, Investigation. **Antonio Rapacciuolo:** Visualization, Investigation. **Giovanni Esposito:** Supervision.

Declaration of competing interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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Prognostic Implications of Declining Hemoglobin Content in Patients Hospitalized With Acute Coronary Syndromes

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ABSTRACT

BACKGROUND Contemporary definitions of bleeding endpoints are restricted mostly to clinically overt events. Whether hemoglobin drop per se, with or without overt bleeding, adversely affects the prognosis of patients with acute coronary syndrome (ACS) remains unclear.

OBJECTIVES The aim of this study was to examine in the MATRIX (Minimizing Adverse Haemorrhagic Events by Transradial Access Site and Systemic Implementation of Angiox) trial the incidence, predictors, and prognostic implications of in-hospital hemoglobin drop in patients with ACS managed invasively stratified by the presence of in-hospital bleeding.

METHODS Patients were categorized by the presence and amount of in-hospital hemoglobin drop on the basis of baseline and nadir hemoglobin values and further stratified by the occurrence of adjudicated in-hospital bleeding. Hemoglobin drop was defined as minimal (<3 g/dl), minor (≥3 and <5 g/dl), or major (≥5 g/dl). Using multivariate Cox regression, we modeled the association between hemoglobin drop and mortality in patients with and without overt bleeding.

RESULTS Among 7,781 patients alive 24 h after randomization with available hemoglobin data, 6,504 patients (83.6%) had hemoglobin drop, of whom 5,756 (88.5%) did not have overt bleeding and 748 (11.5%) had overt bleeding. Among patients without overt bleeding, minor (hazard ratio [HR]: 2.37; 95% confidence interval [CI]: 1.32 to 4.24; p = 0.004) and major (HR: 2.58; 95% CI: 0.98 to 6.78; p = 0.054) hemoglobin drop were independently associated with higher 1-year mortality. Among patients with overt bleeding, the association of minor and major hemoglobin drop with 1-year mortality was directionally similar but had wider CIs (minor: HR: 3.53 [95% CI: 1.06 to 11.79]; major: HR: 13.32 [95% CI: 3.01 to 58.98]).

CONCLUSIONS Among patients with ACS managed invasively, in-hospital hemoglobin drop ≥3 g/dl, even in the absence of overt bleeding, is common and is independently associated with increased risk for 1-year mortality. (Minimizing Adverse Haemorrhagic Events by Transradial Access Site and Systemic Implementation of Angiox; NCT01433627) (J Am Coll Cardiol 2021;77:375–88) © 2021 by the American College of Cardiology Foundation.

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ABBREVIATIONS AND ACRONYMS

ACE = angiotensin-converting enzyme

ACS = acute coronary syndrome(s)

BARC = Bleeding Academic Research Consortium

CI = confidence interval

eGFR = estimated glomerular filtration rate

HR = hazard ratio

OR = odds ratio

PCI = percutaneous coronary intervention

STEACS = ST-segment elevation acute coronary syndrome

Bleeding events have been extensively associated with higher mortality rates in patients with cardiovascular diseases, including patients with acute coronary syndromes (ACS) and those undergoing coronary revascularization (1,2); therefore, their accurate definition and quantification as endpoints are essential. In the context of cardiovascular randomized controlled trials, most contemporary classifications of bleeding endpoints have been restricted to clinically evident (i.e., overt) bleeding, using thresholds of 3 to 5 g/dl hemoglobin reductions to grade their severity (1–4).

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It is unclear, however, whether a hemoglobin reduction per se, in patients without overt bleeding, is independently associated with mortality and, if so, if this association is quantitatively similar to that observed in patients with overt bleeding. Using data from the MATRIX (Minimizing Adverse Haemorrhagic Events by Transradial Access Site and Systemic Implementation of Angiox) trial of patients with ACS managed invasively (5,6), we examined the incidence, predictors, effects on randomized treatments, and prognostic implications of in-hospital hemoglobin drop in patients with and without adjudicated overt bleeding.

METHODS

MATRIX PROGRAM DESIGN. MATRIX (NCT01433627) was a program of 3 nested randomized controlled trials enrolling a total of 8,404 all-comer patients with ACS, with or without ST-segment elevation, receiving invasive management (7). The study protocol of the MATRIX trial was approved by the institutional ethics committees of participating institutions and the central regulatory body of each of the centers and was conducted according to the Declaration of Helsinki and Good Clinical Practice. Detailed study design, methods, and enrollment criteria have been previously published (7). In brief, the first study, MATRIX Access, randomized 8,404 patients with ACS to radial access or femoral access (5). The second study, MATRIX Antithrombin, randomized 7,213 patients comparing an antithrombotic strategy of bivalirudin versus unfractionated heparin (with optional glycoprotein IIb/IIIa inhibitors) (8). The third study, MATRIX Treatment Duration, randomized patients assigned to bivalirudin to receive extended bivalirudin administration after percutaneous coronary intervention (PCI) or short-term administration during PCI (8). Patients with ST-segment elevation ACS were eligible if they presented within 12 h of symptom onset or between 12 and 24 h with evidence of continuing ischemia or previous fibrinolytic treatment and if they had ST-segment elevation ≥1 mm in 2 or more contiguous electrocardiographic leads or a new left bundle branch block or true posterior myocardial infarction. Patients with non-ST-segment elevation ACS were eligible if they had histories consistent with new or worsening cardiac ischemia, occurring at rest or with minimal activity within 7 days before randomization and fulfilled at least 2 high-risk criteria among the following: age ≥60 years, elevation of cardiac biomarkers, electrocardiographic changes consistent with cardiac ischemia, and consideration as possible candidates for PCI after completion of coronary angiography (7).

STUDY PATIENTS AND DEFINITIONS. For this analysis, the study population included all patients enrolled in the MATRIX program who: 1) did not experience fatal events during the first 24 h after randomization, to minimize the risk for immortal time bias; and 2) had qualifying in-hospital hemoglobin information available, including values at baseline and nadir, which were prospectively collected in the electronic care report form. Baseline hemoglobin was the first hemoglobin value obtained at admission and prior to randomization. Nadir hemoglobin was the lowest value collected during hospitalization. All laboratory values, including in-hospital hemoglobin, were entered by the site and then locally monitored and centrally verified for quality using consistency checks. We defined patients as with hemoglobin drop those with positive differences between baseline and nadir hemoglobin values (i.e., the baseline was higher than the nadir). If this difference was zero or negative (i.e., the baseline was equal or lower than the nadir), patients were categorized into the no hemoglobin drop group. Patients with in-hospital hemoglobin drop were then further

The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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stratified on the basis of the presence or absence of an adjudicated overt bleeding during hospitalization. According to contemporary definitions of bleeding (3,4), thresholds of 3 and 5 g/dl were used to classify hemoglobin drop severity. A reduction was considered minimal if the difference between baseline and nadir hemoglobin concentrations was >0 and <3 g/dl, minor if this difference was ≥3 and <5 g/dl, and major if ≥5 g/dl.

STUDY ENDPOINTS. The primary endpoint for this analysis was all-cause mortality occurring from 24 h to 1 year from randomization. As secondary endpoint, all-cause mortality from 24 h to 30 days was also evaluated. An independent clinical events committee blinded to randomized treatment allocation adjudicated all suspected primary or secondary outcomes, including death and bleeding, by reviewing relevant medical records after site monitoring, and systematically identified potential bleeding events, either reported or not by the investigators, in patients with and without hemoglobin reduction. Specifically, all patients with in-hospital hemoglobin drop of at least 3 g/dl were centrally triggered, and source documentation was submitted to the clinical events committee for potential unreported bleeding events.

STATISTICAL ANALYSIS. Differences across groups were assessed using Student's *t*-test or the Wilcoxon-Mann-Whitney *U* test for continuous variables and the chi-square or Fisher exact test for categorical data. The incidence, distribution, and degree of hemoglobin reduction in the study population were assessed. Independent predictors of hemoglobin drop and Bleeding Academic Research Consortium (BARC) type 3 or 5 bleeding were selected with multivariate stepwise selection ($p < 0.01$), starting with forward selection and followed by backward selection. We applied a multivariate Cox proportional hazards model to evaluate the association of hemoglobin drops during index hospitalization (as a categorical and a continuous variable) with all-cause mortality from 24 h to 1 year from randomization in patients with and without overt bleeding. Patients without hemoglobin drop and no overt bleeding were the reference category to compute hazard ratios (HRs) for those with hemoglobin drop without overt bleeding. Patients without hemoglobin drop who bled were used as reference for the hemoglobin drop and overt bleeding group. Covariates tested in the model were chosen among the previously validated predictors of death in ACS populations, including those reported in the GRACE (Global Registry of Acute Coronary Events) risk score (9). The final multivariate model included the following covariates: age, heart rate, systolic blood pressure, estimated glomerular filtration rate, and baseline hemoglobin values as continuous variables; sex, diabetes, cardiac arrest on admission, Killip class, prior myocardial infarction, ST-segment elevation at presentation, and number of diseased coronary vessels (1, 2, or ≥3) as categorical variables. Continuous relation between hemoglobin drop and mortality was assessed using restricted cubic splines. The Kaplan-Meier method was used to estimate cumulative rates of events from 24 h to 30 days and 1 year of follow-up. Waterfall plots were used to graphically illustrate the distribution of hemoglobin drop in patients with and without overt bleeding. We performed an additional analysis according to the prespecified randomization subgroups to estimate possible effects of radial access versus femoral access and bivalirudin versus heparin on: 1) in-hospital bleeding and; 2) minor or major hemoglobin drop. We also evaluated the association of in-hospital hemoglobin drops with and without overt bleeding with blood transfusions as endpoint and sensitivity analyses in patients without known anemia at baseline. The analyses were done using Stata release 14.1 (StataCorp, College Station, Texas) and R (R Foundation for Statistical Computing, Vienna, Austria).

RESULTS

Among the 8,404 patients enrolled in the MATRIX trial, 568 (6.7%) were excluded because of incomplete hemoglobin values (baseline, nadir, or both) and 55 (0.6%) because they died within 24 h of randomization. Of the 7,781 patients alive at 24 h with complete hemoglobin information, 83.6% (n = 6,504) experienced hemoglobin drop during the index hospitalization, and 16.4% (n = 1,277) did not. Baseline characteristics, procedural data, and medications of patients with and without hemoglobin drop, as well as of those with missing hemoglobin values, are reported in Supplemental Tables 1 to 3. Unadjusted mortality rates of patients with or without qualifying hemoglobin values are shown in Supplemental Figure 1, indicating higher in-hospital mortality for patients with incomplete compared with complete hemoglobin information.

Of the 6,504 patients with in-hospital hemoglobin drop, 748 (11.5%) had at least 1 adjudicated overt bleeding event. Baseline characteristics, procedural data, and medications at discharge of patients with and without hemoglobin drop stratified by the presence or absence of overt bleeding are shown in **Table 1** and Supplemental Tables 4 and 5. Among patients with no hemoglobin reduction, patients who bled were older, more frequently had histories

TABLE 1 Baseline Characteristics of Patients With and Without Hemoglobin Drop Stratified by the Presence or Absence of Overt Bleeding

	Hemoglobin Drop Without Overt Bleeding (n = 5,756)	Hemoglobin Drop With Overt Bleeding (n = 748)	p Value	No Hemoglobin Drop Without Overt Bleeding (n = 1,178)	No Hemoglobin Drop With Overt Bleeding (n = 99)	p Value
Age, yrs	65.2 ± 11.7	68.4 ± 11.7	<0.001	66.2 ± 11.9	69.5 ± 11.3	0.007
≥75 yrs	1,368 (23.8)	273 (36.5)	<0.001	335 (28.4)	37 (37.4)	0.078
Male	4,298 (74.7)	509 (68.0)	<0.001	857 (72.8)	78 (78.8)	0.23
Weight, kg	77.6 ± 14	74.7 ± 13.7	<0.001	77.5 ± 13.2	74.3 ± 12.9	0.014
Body mass index, kg/m^2	27.1 ± 4.2	26.5 ± 4	<0.001	27.2 ± 4.1	26.5 ± 3.5	0.068
≥25 kg/m^2	3,865 (67.1)	460 (61.5)	0.002	814 (69.1)	65 (65.7)	0.55
Diabetes mellitus	1,279 (22.2)	181 (24.2)	0.24	293 (24.9)	17 (17.2)	0.11
Insulin dependent	310 (5.4)	46 (6.1)	0.20	72 (6.1)	4 (4.0)	0.092
Current smoking	2,101 (36.5)	219 (29.3)	<0.001	344 (29.2)	30 (30.3)	0.90
Hypercholesterolemia	2,535 (44.0)	330 (44.1)	>0.99	521 (44.2)	46 (46.5)	0.74
Hypertension	3,629 (63.0)	501 (67.0)	0.039	745 (63.2)	67 (67.7)	0.44
Family history of coronary artery disease	1,552 (27.0)	203 (27.1)	0.95	333 (28.3)	28 (28.3)	>0.99
Previous myocardial infarction	790 (13.7)	87 (11.6)	0.12	212 (18.0)	27 (27.3)	0.032
Previous PCI	812 (14.1)	80 (10.7)	0.013	197 (16.7)	20 (20.2)	0.45
Radial access	121 (2.1)	17 (2.3)	0.013	46 (3.9)	3 (3.0)	0.45
Femoral access	414 (7.2)	24 (3.2)		82 (7.0)	9 (9.1)	
Both radial and femoral access	50 (0.9)	2 (0.3)		16 (1.4)	2 (2.0)	
Access site unknown	227 (3.9)	37 (4.9)		53 (4.5)	6 (6.1)	
Previous CABG	150 (2.6)	24 (3.2)	0.40	62 (5.3)	9 (9.1)	0.17
Previous transient ischemic attack or stroke	265 (4.6)	47 (6.3)	0.053	68 (5.8)	5 (5.1)	0.94
Peripheral vascular disease	442 (7.7)	101 (13.5)	<0.001	112 (9.5)	12 (12.1)	0.50
Chronic obstructive pulmonary disease	357 (6.2)	57 (7.6)	0.15	72 (6.1)	10 (10.1)	0.18
Renal failure	70 (1.2)	10 (1.3)	0.91	17 (1.4)	2 (2.0)	0.65
Dialysis	4 (0.1)	1 (0.1)	0.45	3 (0.3)	0 (0.0)	>0.99
Cardiac arrest	108 (1.9)	26 (3.5)	0.006	21 (1.8)	0 (0.0)	0.40
Killip class			<0.001			0.40
I	5,238 (91)	643 (86)		1,077 (91.4)	89 (89.9)	
II	372 (6.5)	65 (8.7)				
III	109 (1.9)	22 (2.9)		1,077 (91.4)	89 (89.9)	
IV	37 (0.6)	18 (2.4)				
Previous lytic therapy	152 (2.6)	10 (1.3)	0.043	22 (1.9)	2 (2.0)	0.70
STEACS	2,915 (50.6)	420 (56.1)	0.005	367 (31.2)	33 (33.3)	0.73
NSTEACS	2,841 (49.4)	328 (43.9)	0.005	811 (68.8)	66 (66.7)	
NSTEACS, troponin negative	323 (5.6)	29 (3.9)	0.059	97 (8.2)	6 (6.1)	0.56
Systolic arterial pressure, mm Hg	139 ± 25.3	141.4 ± 29.5	0.038	138.2 ± 24.6	134.3 ± 26.4	0.22
Heart rate, beats/min	76.4 ± 16.6	77.4 ± 18.5	0.15	74.7 ± 16.1	74.4 ± 15.8	0.84
Left ventricular ejection fraction, %	51.2 ± 9.5	49.8 ± 10.5	0.001	51.2 ± 9.6	49.9 ± 10.9	0.28
Hemoglobin, g/dl	14.2 ± 1.7	13.9 ± 1.9	<0.001	13 ± 1.8	13 ± 2	0.83
eGFR, ml/min/1.73 m^2	84 ± 25.3	78.6 ± 24.9	<0.001	85.5 ± 26.5	80.3 ± 23.6	0.07
<60 ml/min/1.73 m^2	929 (16.2)	175 (23.5)	<0.001	198 (16.8)	23 (23.2)	0.14
<30 ml/min/1.73 m^2	55 (1.0)	7 (0.9)	>0.99	13 (1.1)	0 (0.0)	0.61

Continued on the next page

of myocardial infarction, and more often received intra-aortic balloon pump support than patients without overt bleeding. Compared with patients without hemoglobin drop or overt bleeding, patients with both hemoglobin drop and bleeding were older, had more comorbidities and cardiovascular risk factors at presentation (i.e., higher risk profile), and more often received clopidogrel at discharge. Baseline, procedural, and treatment characteristics of patients stratified by hemoglobin drop levels are detailed in Supplemental Tables 6 to 8. The distribution of hemoglobin drop in patients with and without overt bleeding is presented in Supplemental Figure 2. The proportion of patients with minor or major hemoglobin drop was higher among those with overt bleeding (15.1% and 3.5%, respectively) compared with those without overt bleeding (4.7% and 1.1%, respectively; p < 0.001) (**Figure 1, Table 2**). In contrast, a minor or major hemoglobin drop without overt bleeding was observed in 399 patients compared with 158 patients with the same level of hemoglobin drop and concomitant overt bleeding

TABLE 1 Continued

	Hemoglobin Drop Without Overt Bleeding (n = 5,756)	Hemoglobin Drop With Overt Bleeding (n = 748)	p Value	No Hemoglobin Drop Without Overt Bleeding (n = 1,178)	No Hemoglobin Drop With Overt Bleeding (n = 99)	p Value
Medications administered before catheterization						
Aspirin	5,428 (94.3)	713 (95.3)	0.29	1,097 (93.1)	96 (97.0)	0.20
Clopidogrel	2,706 (47)	336 (44.9)	0.29	625 (53.1)	56 (56.6)	0.57
Prasugrel	703 (12.2)	97 (13.0)	0.59	94 (8.0)	10 (10.1)	0.58
Ticagrelor	1,331 (23.1)	194 (25.9)	0.097	301 (25.6)	25 (25.3)	>0.99
Enoxaparin	866 (15.0)	122 (16.3)	0.39	283 (24.0)	31 (31.3)	0.13
Fondaparinux	561 (9.7)	72 (9.6)	0.96	163 (13.8)	17 (17.2)	0.44
ACE inhibitors	1,659 (28.8)	210 (28.1)	0.70	427 (36.2)	39 (39.4)	0.60
Angiotensin II receptor blockers	592 (10.3)	87 (11.6)	0.28	146 (12.4)	16 (16.2)	0.35
Statins	2,416 (42.0)	293 (39.2)	0.15	611 (51.9)	54 (54.5)	0.68
Beta-blockers	2,292 (39.8)	267 (35.7)	0.033	575 (48.8)	47 (47.5)	0.88
Warfarin	89 (1.5)	12 (1.6)	>0.99	25 (2.1)	2 (2.0)	>0.99
Proton pump inhibitors	2,896 (50.3)	385 (51.5)	0.57	683 (58.0)	63 (63.6)	0.32
Unfractionated heparin	1,742 (30.3)	265 (35.4)	0.005	288 (24.4)	25 (25.3)	0.95
Bivalirudin	3 (0.1)	0 (0.0)	>0.99	0 (0.0)	0 (0.0)	>0.99
Glycoprotein IIb/IIIa inhibitors	10 (0.2)	2 (0.3)	0.64	1 (0.1)	1 (1.0)	0.14

Values are mean ± SD or n (%). The chi-square or Fisher exact test was used for categorical variables; Student's *t*-test or the Wilcoxon test was used for continuous variables.

ACE = angiotensin-converting-enzyme; CABG = coronary artery bypass graft; eGFR = estimated glomerular filtration rate; NSTEACS = non-ST-segment elevation acute coronary syndrome; PCI = percutaneous coronary intervention; STEACS = ST-segment elevation acute coronary syndrome.

(Table 2). Among patients with overt bleeding, 36.8% had BARC type 2 bleeding, while 16.4% had BARC type 3 or 5 bleeding (Supplemental Table 9). The percentage changes in hemoglobin levels in patients with overt bleeding stratified by BARC type severity (type 1 or 2 vs. type 3, 4, or 5) is reported in Supplemental Figure 3.

PREDICTORS OF MAJOR OR MINOR HEMOGLOBIN DROP AND BARC TYPE 3 OR 5 BLEEDING. To comprehensively characterize the potential use of hemoglobin drop as surrogate, multivariate predictors at baseline of major or minor hemoglobin drop as well as of BARC type 3 or 5 type bleeding were assessed (Table 3). Predictors of major or minor hemoglobin drop included age, female sex, body mass index, diabetes, non-ST-segment elevation ACS presentation, heart rate, clopidogrel on admission, and Killip class at presentation. Notably, baseline hemoglobin independently predicted both major or minor hemoglobin drop and BARC type 3 or 5 bleeding but with a directionally opposite association.

MULTIVARIATE ASSOCIATION OF HEMOGLOBIN DROP WITH ALL-CAUSE MORTALITY. Kaplan-Meier event curves showed a higher cumulative incidence of mortality from 24 h to 1 year in patients with major or minor hemoglobin drop compared with those with minimal or no hemoglobin drop, with a similar pattern in patients with and without overt bleeding (Figure 2). Multivariate association of hemoglobin drop with 30-day and 1-year mortality in patients with and without overt bleeding is reported in Table 4 and displayed in Figure 3 and the Central Illustration. In general, hemoglobin drop in patients with and without overt bleeding was independently associated with a graded association with mortality, which was higher for patients with overt bleeding compared with those without overt bleeding. Yet patients without overt bleeding had a higher risk for 1-year mortality after a minor (HR: 2.37; 95% confidence interval [CI]: 1.32 to 4.24; p = 0.004) or a major (HR: 2.58; 95% CI: 0.98 to 6.78, p = 0.054) hemoglobin drop, respectively, compared with those without hemoglobin drop and without overt bleeding. Among patients with overt bleeding, the hazard of 1-year mortality for minor and major hemoglobin drop, compared with patients without hemoglobin drop, was directionally similar but had wider CIs (minor: HR: 3.53 [95% CI: 1.06 to 11.79; p = 0.040]; major: HR: 13.32 [95% CI: 3.01 to 58.98; p = 0.001]). The multivariate 1-year HR for mortality of hemoglobin drop modeled as a continuous variable was 1.18 per g/dl decrease in hemoglobin for patients without overt bleeding (95% CI: 1.04 to 1.34; p = 0.010) and 1.41 for patients with overt bleeding (95% CI: 1.19 to 1.67; p < 0.001). Results were directionally similar at 30 days as well as restricting the population to those without known anemia at baseline (excluding 1,536 patients with baseline anemia) (Supplemental Table 10).

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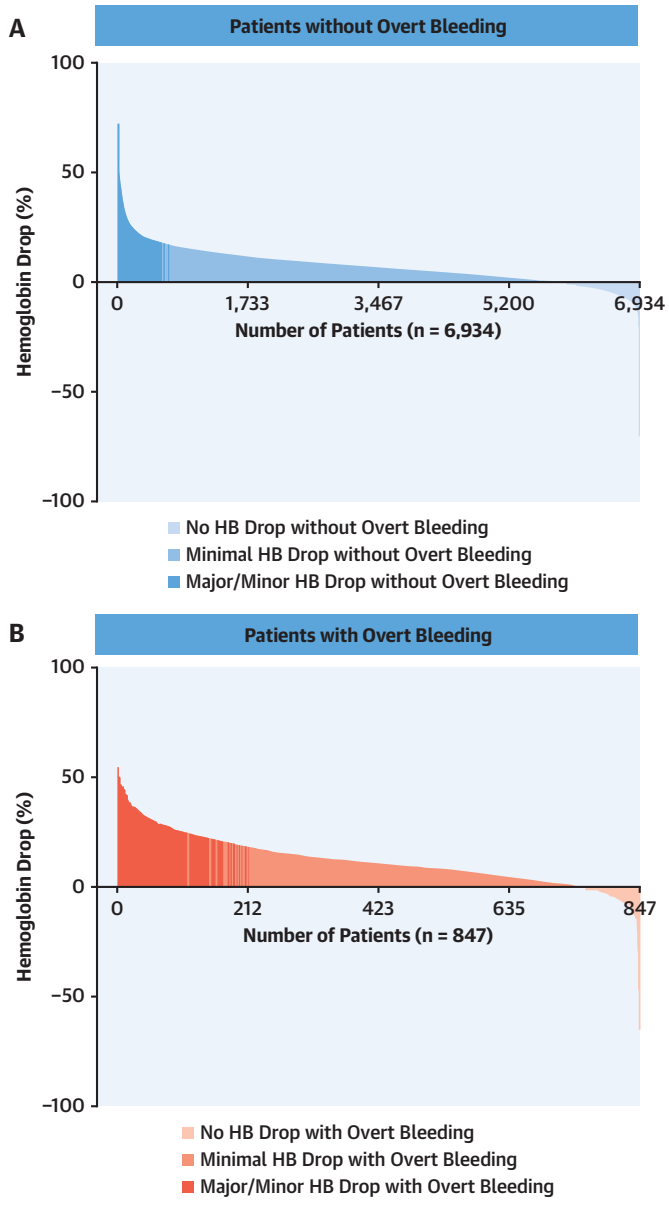
FIGURE 1 Waterfall Plot for the Percentage Change in Hemoglobin Level During Hospitalization



(A) Patients without overt bleeding. **(B)** Patients with overt bleeding. Data are shown for patients alive 24 h after randomization who had no hemoglobin (Hb) reduction **(light blue and light red)**, minimal hemoglobin reduction **(blue and red)**, and major or minor hemoglobin reduction **(dark blue and dark red)**. The y-axis shows percentage of hemoglobin drop. The x-axis shows absolute number of patients per group.

EFFECTS OF RANDOMIZED TREATMENTS ON HEMOGLOBIN DROP WITH OR WITHOUT BLEEDING. The effect of randomized treatments on in-hospital overt bleeding and in-hospital minor or major hemoglobin drop is reported in Table 5. The use of radial over femoral access was associated with a lower risk for in-hospital bleeding complications (odds ratio [OR]: 0.51; 95% CI: 0.44 to 0.59; $p < 0.001$) and a numerically lower risk for minor or major hemoglobin reduction (OR: 0.85; 95% CI: 0.71 to 1.01; $p = 0.065$). The use of bivalirudin was associated with a significantly reduced risk for in-hospital bleeding (OR: 0.77; 95% CI: 0.67 to 0.89; $p < 0.001$) and minor or major hemoglobin reduction (OR: 0.78; 95% CI: 0.64 to 0.94; $p = 0.010$) compared with unfractionated heparin. The rates and proportions of patients with and without hemoglobin reduction receiving blood transfusions are reported in Supplemental Table 11.

TABLE 2 Grade of Hemoglobin Drop in Patients With and Without Overt Bleeding

	Patients Without Overt Bleeding (n = 6,934)	Patients With Overt Bleeding (n = 847)	p Value
No hemoglobin drop	1,178 (17)	99 (11.7)	<0.001
Minimal hemoglobin drop (<3 g/dl)	5,357 (77.3)	590 (69.7)	
Minor hemoglobin drop (≥3 and <5 g/dl)	325 (4.7)	128 (15.1)	
Major hemoglobin drop (≥5 g/dl)	74 (1.1)	30 (3.5)	

Values are n (%). The chi-square or Fisher exact test was used for categorical variable; Student's *t*-test or the Wilcoxon test was used for continuous variables.

DISCUSSION

In the present analysis, we comprehensively assessed, in an all-comer population of patients with ACS managed invasively, the epidemiology, predictors, and association with outcome of in-hospital hemoglobin drop, with and without overt bleeding. The main findings are the following.

First, an in-hospital hemoglobin drop of ≥3 g/dl, even in the absence of adjudicated overt bleeding, showed a continuous, direct association with increased 1-year mortality. No independent association between minimal (<3 g/dl) hemoglobin drop and mortality was observed.

Second, patients with hemoglobin reduction ≥3 g/dl were proportionally more common (19% vs. 6%) in the group with adjudicated bleeding. Yet the prevalence of hemoglobin reduction ≥3 g/dl in patients without adjudicated bleeding was far higher than among patients with adjudicated bleeding (n = 158 vs. n = 399).

Third, randomized bleeding minimization strategies tested in MATRIX (i.e., radial access and bivalirudin use) were associated with a lower risk for incurring in-hospital hemoglobin drop compared with their control (i.e., femoral access on unfractionated heparin).

POSSIBLE CAUSES AND CONSEQUENCES OF HEMOGLOBIN REDUCTIONS IN PATIENTS WITH ACS. In patients with ACS, multiple mechanisms may be responsible for in-hospital hemoglobin drop. Overt bleeding can

TABLE 3 Multivariate Predictors of Major or Minor Hemoglobin Drop and BARC Type 3 or 5 Bleeding

	Major/Minor HB Drop*		BARC Type 3 or 5 Bleeding*	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Age (for each increase of 10 yrs)	1.31 (1.20–1.42)	<0.001	1.54 (1.29–1.83)	<0.001
Female sex	1.52 (1.22–1.90)	<0.001	–	–
Body mass index (for each increase of 1 kg/m^2)	0.96 (0.94–0.99)	0.001	–	–
Peripheral vascular disease	–	–	2.46 (1.58–3.82)	<0.001
Diabetes mellitus	1.28 (1.01–1.63)	0.047	–	–
Insulin-dependent diabetes mellitus	2.10 (1.48–2.98)	<0.001	–	–
Killip class II (vs. class I)	1.45 (1.07–1.99)	0.020	–	–
Killip class III (vs. class I)	1.43 (0.83–2.48)	0.17	–	–
Killip class IV (vs. class I)	3.43 (1.83–6.43)	<0.001	–	–
NSTEACS on admission	0.63 (0.52–0.76)	<0.001	0.49 (0.34–0.70)	<0.001
Troponin-negative NSTEACS	0.43 (0.22–0.82)	0.011	–	–
Hemoglobin at baseline (for each increase of 1 g/dl)	1.51 (1.41–1.60)	<0.001	0.82 (0.75–0.90)	<0.001
Heart rate (for each increase of 10 beats/min)	1.11 (1.06–1.17)	<0.001	–	–
Clopidogrel on admission	0.76 (0.63–0.92)	0.005	0.57 (0.39–0.83)	0.003

*Multivariate analysis included all patients with complete hemoglobin data (n = 7,806).

BARC = Bleeding Academic Research Consortium; CI = confidence interval; HB = hemoglobin; NSTEACS = non-ST-segment elevation acute coronary syndrome; OR = odds ratio.

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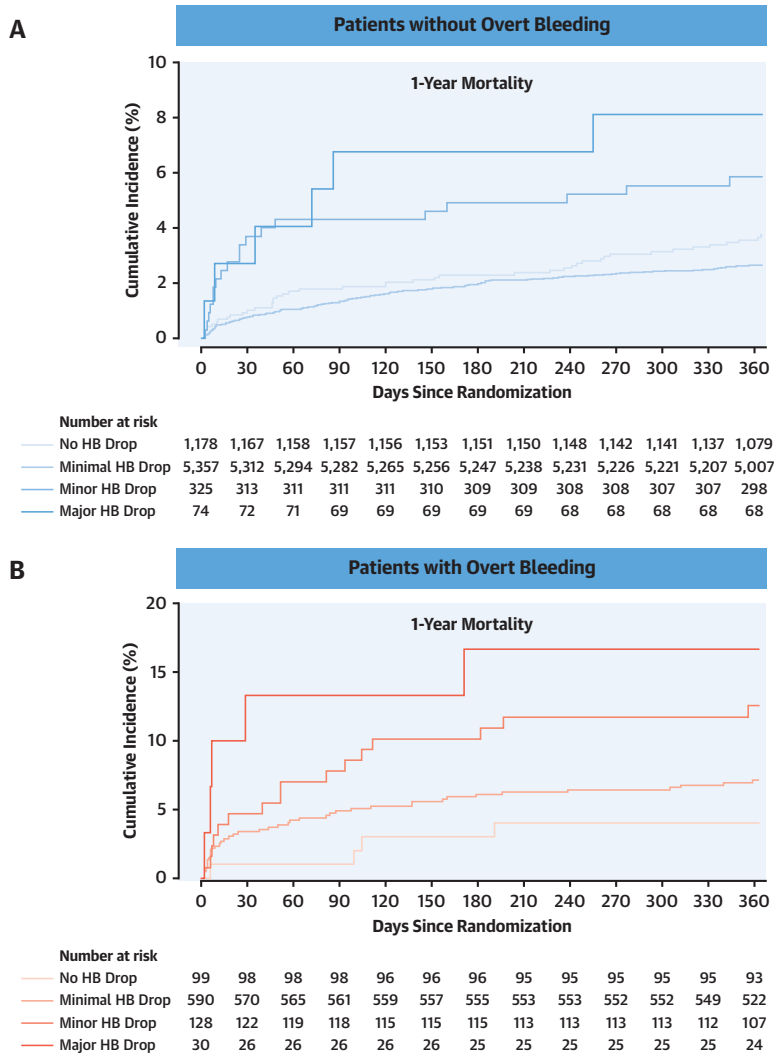
FIGURE 2 1-Year Mortality in Patients With and Without Overt Bleeding



Kaplan-Meier event curves for all-cause mortality stratified by hemoglobin (Hb) drop from 24 h to 1 year. **(A)** Patients without overt bleeding. **(B)** Patients with overt bleeding.

complicate hospital course as a consequence of pharmacological as well as invasive procedures (10,11). Besides evident blood loss, subtle bleeding can also occur because of the aggressive antithrombotic burden, with the primary source of bleeding remaining masked if not investigated appropriately. Also, a decline in hemoglobin after ACS can be caused by blood loss during the index procedure, intense inflammatory status (12), stress polycythemia on admission (13), hemodilution secondary to volume

TABLE 4 Multivariate Association With 30-Day and 1-Year All-Cause Mortality of In-Hospital Hemoglobin Reduction With and Without Overt Bleeding

	30-Day Mortality HR (95% CI)	p Value	1-Year Mortality HR (95% CI)	p Value
Patients without overt bleeding				
No hemoglobin drop*	Reference	—	Reference	—
Minimal hemoglobin drop (<3 g/dl)	0.78 (0.40–1.56)	0.48	0.85 (0.60–1.22)	0.39
Minor hemoglobin drop (≥3 and <5 g/dl)	4.30 (1.71–10.78)	0.002	2.37 (1.32–4.24)	0.004
Major hemoglobin drop (≥5 g/dl)	3.39 (0.68–16.85)	0.13	2.58 (0.98–6.78)	0.054
Continuous hemoglobin drop (for each increase of 1 g/dl)†	1.41 (1.16–1.70)	0.001	1.18 (1.04–1.34)	0.010
Patients with overt bleeding				
No hemoglobin drop‡	Reference	—	Reference	—
Minimal hemoglobin drop (<3 g/dl)	4.19 (0.52–34.04)	0.18	2.41 (0.82–7.08)	0.11
Minor hemoglobin drop (≥3 and <5 g/dl)	3.22 (0.33–31.63)	0.31	3.53 (1.06–11.79)	0.040
Major hemoglobin drop (≥5 g/dl)	33.8 (2.84–402.35)	0.005	13.32 (3.01–58.98)	0.001
Continuous hemoglobin drop (for each increase of 1 g/dl)†	1.44 (1.13–1.84)	0.003	1.41 (1.19–1.67)	<0.001

*The reference group consisted of patients without hemoglobin drop and without overt bleeding. †If reduction <0 g/dl, consider 0 g/dl. ‡The reference group consisted of patients without hemoglobin drop and with overt bleeding.

CI = confidence interval; HR = hazard ratio.

repletion (14), or impaired bone marrow activity due to clinical factors (15).

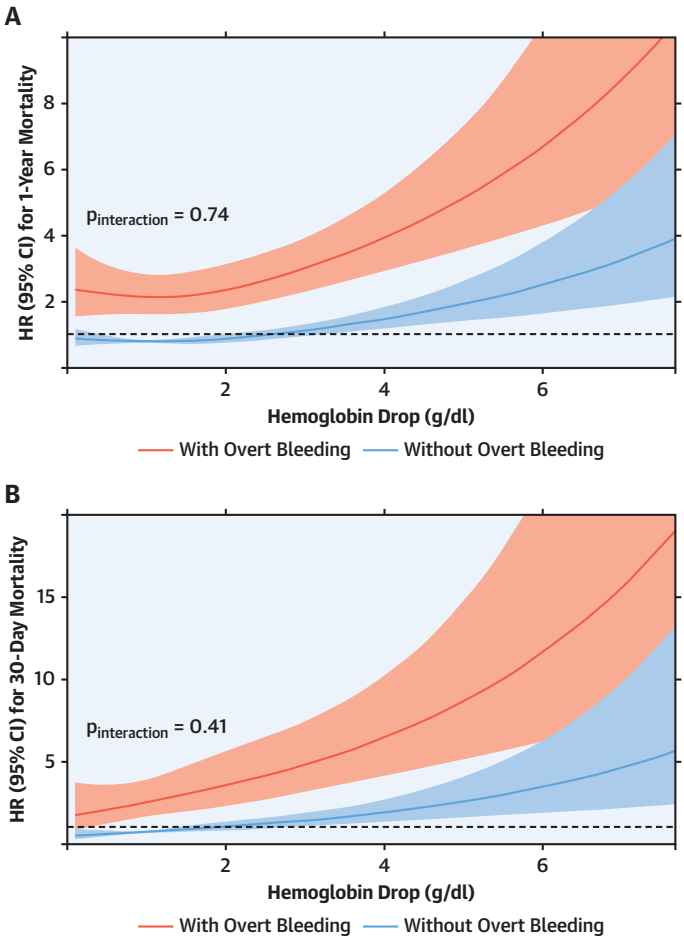
In the early phase of ACS, anemia has been consistently associated with bleeding complications (16,17). However, its prognostic impact can also extend to nonbleeding outcomes and mortality (18–20), possibly by worsening myocardial ischemic insult (i.e., decreasing the oxygen supply to the jeopardized myocardium) (21), increasing myocardial oxygen demand (i.e., need for higher cardiac output to maintain an adequate systemic oxygen delivery) (22), and inducing abnormal neurohormonal activation and cardiac remodeling (23). Previous studies showed that the presence of low hemoglobin levels before and/or after PCI is a powerful and independent predictor of future cardiovascular events (18–20). In a large pooled population of 39,922 patients with ACS, baseline hemoglobin level <11 g/dl was associated with excess mortality of more than 4-fold compared with higher values (18).

Evidence also indicates an adverse prognostic impact for in-hospital hemoglobin changes in this setting. Among 7,608 patients undergoing successful PCI from the ADAPT-DES (Assessment of Dual Antiplatelet Therapy With Drug-Eluting Stents) registry (51.7% presenting with ACS), in-hospital hemoglobin reduction ≥4.0 g/dl in the presence of overt bleeding was associated with considerably increased risk for dying (24). In a prospective study involving 1,390 patients with myocardial infarction (15), in-hospital hemoglobin drop and nadir showed a significant and independent association with 2-year mortality. A relevant proportion of patients showed decreases in hemoglobin levels during hospitalization in the absence of bleeding, with a more than doubled proportion of patients having anemia at discharge (36.1%) than on admission (17.8%). In the TRIUMPH registry (25), including 2,909 patients with ACS and normal hemoglobin levels on admission, up to 45% of patients developed anemia during hospitalization, which if moderate to severe (<11 g/dl in 26% of cases) was associated with worse mortality and health status at 1 year. Among patients who developed in-hospital anemia, 86% did not have overt bleeding. Finally, post-PCI drop in hemoglobin levels has been associated with acute kidney injury (26), which in turn has demonstrated to be a relevant driver of mortality in the setting of ACS (27,28).

However, no previous study addressed the prognostic impact of hemoglobin reduction per se (i.e., without concomitant overt bleeding) in the setting of ACS.

PROGNOSTIC EFFECT OF HEMOGLOBIN REDUCTIONS IN PATIENTS WITH ACS WITHOUT OVERT BLEEDING AND IMPLICATIONS FOR BLEEDING ENDPOINT CLASSIFICATIONS. The present analysis extends previous evidence by examining the incidence and prognostic relevance of hemoglobin drop, with and without concomitant overt bleeding, in a large contemporary ACS population. In line with previous findings (15,25), the incidence of hemoglobin reduction was high in our population. In patients without adjudicated bleeding, a heightened risk for mortality was apparent at a hemoglobin threshold of approximately 3 g/dl. Thus, in the absence of overt bleeding, a minor (between 3 and 5 g/dl) or major (more than 5 g/dl) hemoglobin drop was associated with an increase in the risk for dying of about 2.5-fold at 1 year, which was independent of

FIGURE 3 Spline Functions of 1-Year and 30-Day Mortality in Patients With and Without Overt Bleeding According to In-Hospital Hemoglobin Drop

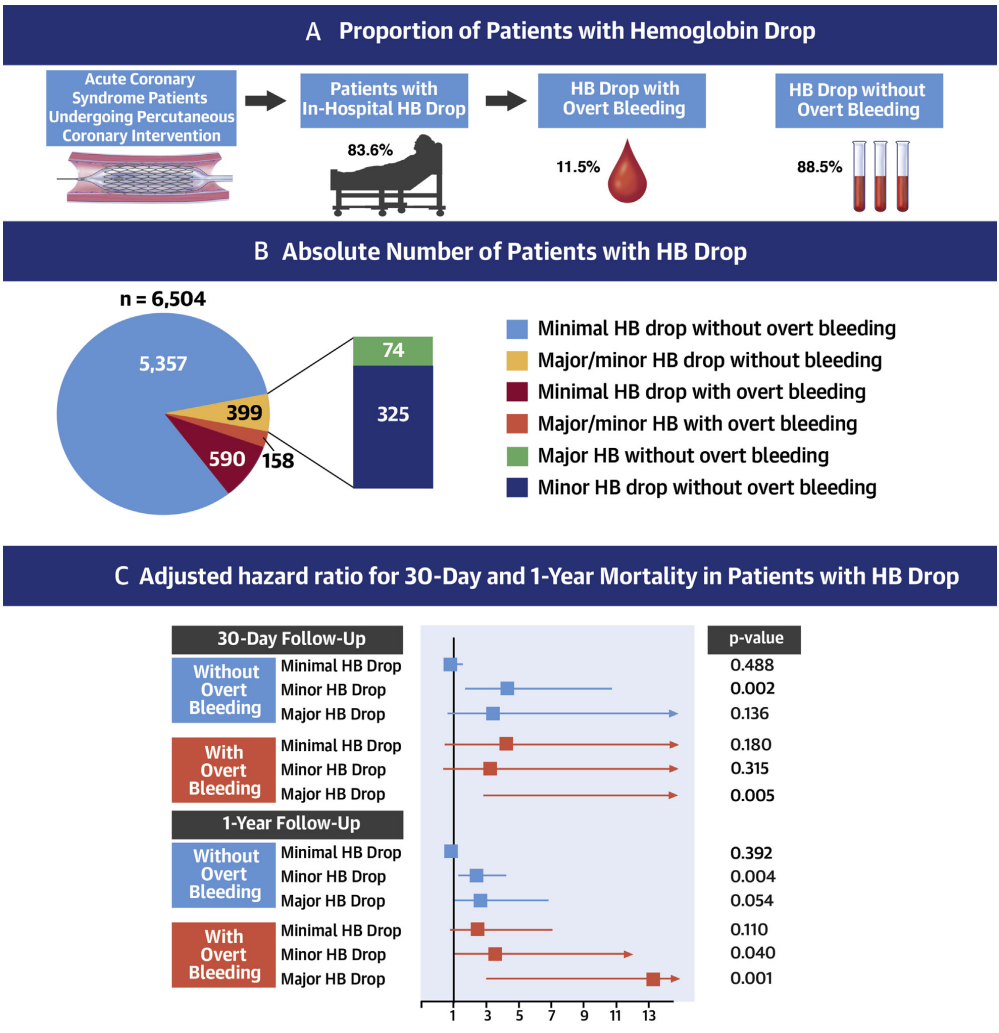


(A) Adjusted mortality risk from 24 h to 1 year. **(B)** Adjusted mortality risk from 24 h to 30 days. **Red** indicates patients with overt bleeding; **blue** indicates patients without overt bleeding. CI = confidence interval; HR = hazard ratio.

several covariates, including clinical and procedural factors and, importantly, baseline hemoglobin concentrations. Conversely, a minimal hemoglobin reduction (<3 g/dl) was not associated with mortality. In contrast, life-threatening bleeding such as intracranial hemorrhage or cardiac tamponade may occur with modest blood loss. This might in part explain why, in patients with adjudicated bleeding, an increase in the risk for mortality in multivariate analysis (although nonsignificant) was apparent even for minimal hemoglobin drop.

Adjudication of bleeding endpoints in most randomized controlled trials according to contemporary definitions mandates the presence of overt bleeding (29,30). This modern approach deviates from historical frameworks, which considered a decrease in hemoglobin as a minor hemorrhagic event even in the absence of overt bleeding (i.e., blood loss with no site

CENTRAL ILLUSTRATION **In-Hospital Hemoglobin Drop in Patients With and Without Overt Bleeding in Acute Coronary Syndrome**



Leonardi, S. et al. J Am Coll Cardiol. 2021;77(4):375–88.

(A) Approximately 8 in 10 patients invasively managed for acute coronary syndrome (ACS) can develop hemoglobin (HB) drop during the index hospitalization. In 7 of these 8 patients, hemoglobin reduction is observed in the absence of overt bleeding. **(B)** Although patients without overt bleeding frequently have a minimal hemoglobin drop (<3 g/dl), in view of the millions of individuals with ACS undergoing invasive management worldwide, a substantial number of patients can experience minor (≥3 g/dl) or major (≥5 g/dl) hemoglobin drop. **(C)** Patients with hemoglobin drop ≥3 g/dl showed doubled risk for 1-year mortality, even in the absence of overt bleeding. HR = hazard ratio; PCI = percutaneous coronary intervention.

TABLE 5 Effect of Radial Versus Femoral Access and of Bivalirudin Versus Unfractionated Heparin on In-Hospital Overt Bleeding and In-Hospital Minor or Major Hemoglobin Drop

	In-Hospital Overt Bleeding OR (95% CI) (n = 8,349)	p Value	In-Hospital Minor or Major Hemoglobin Drop OR (95% CI) (n = 7,781)	p Value
Radial access vs. femoral access	0.51 (0.44–0.59)	<0.001	0.85 (0.71–1.01)	0.065
Bivalirudin vs. unfractionated heparin	0.77 (0.67–0.89)	<0.001	0.78 (0.64–0.94)	0.010

Abbreviations as in Table 3.

identified) (31). Notably, most studies do not require systematic investigation of potential sources of hemoglobin loss. Thus, in clinical practice as well as in clinical research, many potential bleeding events may remain occult. Moreover, bleeding events considered prognostically relevant (such as BARC type 3 to 5) are relatively infrequent (29,30), limiting study power (32).

The observation of a direct, continuous, independent association with long-term mortality as well as a measurable treatment effect on established bleeding minimization strategies supports the concept that a threshold of in-hospital hemoglobin reduction higher than 3 g/dl may serve as a valid surrogate endpoint (33). As such, this level of hemoglobin reduction could complement contemporary definitions of bleeding endpoints and be easily implemented because of the simple, reliable, and inexpensive measurement.

Finally, a strength of this study is the inclusion of baseline hemoglobin as a covariate. The directionally inverse association of baseline hemoglobin with major or minor hemoglobin drop in patients without overt bleeding (vs. those with adjudicated bleeding) likely reflects the definition of hemoglobin drop (i.e., the higher the baseline value, the more likely a hemoglobin drop is observed) and supports the inclusion of baseline hemoglobin in the multivariate model with death as outcome (Table 4).

Therefore, regardless of baseline hemoglobin, a hemoglobin drop ≥3 g/dl, even in the absence of detectable bleeding, appears prognostically relevant.

STUDY LIMITATIONS. This was a post hoc analysis from a prospective, randomized controlled trial, which was not powered to explore outcome differences across hemoglobin reduction subgroups of patients. As such, the results should only be considered hypothesis generating. Qualifying hemoglobin values were missing in 7.1% of patients. Although the reason for this was not captured, the high early mortality rate of these patients indicates that an early fatal event prevented the collection of hemoglobin values in many of them. Specific conditions associated with or predisposing to chronic anemia were not assessed in detail in the study population and might have influenced our results. Another possible limitation is that some misclassification of hemoglobin reduction severity occurred in patients who received blood transfusions during the hospital stay. Finally, in the MATRIX trial, data on hemoglobin at follow-up were not collected. Thus, we were not able to analyze the prognostic impact of transient (i.e., in-hospital only) versus persistent (i.e., after discharge) anemia.

CONCLUSIONS

In patients with ACS managed invasively, in-hospital decreases in hemoglobin levels ≥3 g/dl, even in the absence of overt bleeding events, were common and independently associated with an increased risk for all-cause mortality at 1 year. If confirmed, these results may help the identification of higher risk patients and inform contemporary bleeding definitions.

AUTHOR DISCLOSURES

The MATRIX trial was sponsored by Società Italiana di Cardiologia Invasiva (a nonprofit organization), which received grant support from The Medicines Company and Terumo. This substudy did not receive any direct or indirect funding. Dr. Leonardi has received grants and personal fees from AstraZeneca, Bristol Myers Squibb/Pfizer, and Chiesi; and has received personal fees from Bayer outside the submitted work. Dr. Gragnano has received research grant support from the European Society of Cardiology outside the submitted work. Dr. Gargiulo has received consultant fees from Daiichi-Sankyo outside the submitted work. Dr. Vranckx has received personal fees from AstraZeneca, Terumo, CSL Behring, Daiichi-Sankyo, and Bayer Health Care outside the submitted work. Dr. Frigoli is affiliated with CTU Bern, University of Bern, which has a staff policy of not accepting honoraria or consultancy fees. However, CTU Bern is involved in the design, conduct, or analysis of clinical studies funded by not-for-profit and for-profit organizations. In particular, pharmaceutical and medical device companies provide direct funding to some of these studies. For an up-to-date list of CTU Bern's conflicts of interest, visit the University of Bern Web site (and see Research, Declaration of Interest). Dr. Windecker has received research and educational grants to the institution from Abbott, Amgen, Bristol Myers Squibb, Bayer, Boston Scientific,

Biotronik, Cardinal Health, CSL Behring, Daiichi-Sankyo, Edwards Lifesciences, Johnson & Johnson, Medtronic, Querbet, Polares, Sanofi, Terumo, and Sinomed outside the submitted work. Dr. Valgimigli has received grants and personal fees from Abbott, Terumo, AstraZeneca; has received personal fees from Chiesi, Bayer, Daiichi-Sankyo, Amgen, Alvimedica, Biosensors, and Idorsia; and has received grants from Medicure outside the submitted work. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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PERSPECTIVES

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: In invasively managed patients with ACS, a decrease in hemoglobin content of ≥3 g/dl is associated with 1-year mortality even in the absence of overt bleeding.

TRANSLATIONAL OUTLOOK: A declining hemoglobin level may be a useful surrogate for other bleeding-related endpoints in clinical trials investigating treatment strategies for patients with ACS undergoing invasive management.

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KEY WORDS acute coronary syndromes, bleeding, hemoglobin, percutaneous coronary intervention

APPENDIX For supplemental tables and figures, please see the online version of this paper.

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One-Year Clinical Outcomes of the Legflow Drug-Coated Balloon for the Treatment of Femoropopliteal Occlusions Registry

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Abstract

Purpose: To report the 1-year outcomes of the prospective Legflow drug-coated balloon (DCB) registry, which evaluated the safety and 12-month efficacy of the Legflow balloon in the treatment of femoropopliteal disease. **Methods:** The Legflow is a new generation of DCB that has a homogenous, stable surface coating incorporating 0.1-µm paclitaxel particles. From January 2014 to June 2016, 139 patients (mean age 67.1±10.8 years; 109 men) were enrolled at 4 European institutions. Seventy-nine (56.8%) patients had claudication, while 60 (43.2%) had critical limb ischemia (CLI). Mean lesion length (MLL) was 90.0±41.2 mm. Eighty (57.6%) patients were treated for de novo lesions (MLL 83.2±41.2 mm), 29 (20.9%) for postangioplasty restenosis (MLL 81.2±30.9 mm), and 30 (21.6%) for in-stent restenosis (MLL 117.0±39.5 mm). The primary outcome measure was freedom from binary restenosis as determined by a peak systolic velocity ratio ≥2.4 on duplex or >50% stenosis on digital subtraction angiography at 12 months. The secondary outcome was freedom from clinically-driven target lesion revascularization (CD-TLR) at 12 months. **Results:** Technical success was achieved in all the 139 treated patients. During the hospital stay, 3 CLI patients died of wound-related complications and 3 CLI patients underwent urgent TLR due to early occlusion in 2 and stent thrombosis in 1. At 12 months, 4 additional patients died of cardiac disease unrelated to the procedure. Of the 132 patients available for 1-year follow-up, the primary outcome (freedom from restenosis) was obtained in 107 (81.1%) patients. Freedom from CD-TLR was obtained in 110 (83.3%). Of the 25 late restenoses >50%, only 3 asymptomatic patients did not require TLR. Freedom from CD-TLR was higher in claudicants (87.0%) than in CLI patients (78.2%, p=0.20). In patients treated for in-stent restenosis, freedom from TLR at 1 year was 89.2%. **Conclusion:** These data suggest that the use of a new generation paclitaxel-coated balloon represents a safe and effective therapeutic strategy for femoropopliteal obstructions in different clinical and anatomical settings. These data will need to be confirmed with longer-term follow-up and in randomized controlled trials.

Keywords

balloon angioplasty, drug-coated balloon, drug-eluting balloon, femoropopliteal segment, in-stent restenosis, occlusion, paclitaxel, popliteal artery, stenosis, superficial femoral artery, target lesion revascularization

Introduction

Patients with lower limb atherosclerosis due to femoropopliteal disease may suffer from critical limb ischemia (CLI) or life-limiting claudication and may have multiple comorbidities limiting a surgical option.[1] In these cases, balloon angioplasty is a valuable therapeutic approach, but its long-term efficacy is hampered by restenosis of the treated arterial segment. Use of early generation drug-coated balloons (DCBs) has shown promising results in the prevention and treatment of postangioplasty restenosis in the femoropopliteal segment in randomized clinical trials.[2] The devices

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tested so far have several unsolved technical limitations, such as inconsistent drug coating concentrations, significant drug loss during device tracking to the target lesion, and use of bulky paclitaxel particles. In addition, the elevated initial balloon-artery drug transfer rates resulted in too high early drug-in-tissue concentrations.[3] All these limitations may reduce the therapeutic efficacy of the drug released onto the arterial wall and hamper efficacy in challenging clinical (ie, CLI patients) or anatomical (ie, long lesions, small vessels) situations.

To overcome these limitations, a new generation DCB (Legflow; Cardionovum GmbH, Bonn, Germany) has been developed.[4] The balloon is covered with a homogenous, stable surface composed of 0.1-µm paclitaxel particles embedded in an ammonium salt compound; the excipient prolongs the availability of paclitaxel in the vessel wall, enhancing the antiproliferative effect. The nanocrystalline particles are not on the exterior of the furled balloon, so there is no risk that paclitaxel will flake off during DCB insertion into the sheath or during endovascular tracking to the target lesion.

Currently, there is scant information on the efficacy of this new DCB in the treatment of femoropopliteal obstructions in daily endovascular practice. The RAPID trial,[5] a safety and efficacy study of the Legflow DCB in combination with primary Supera stenting in patients with intermediate to long superficial femoral artery (SFA) lesions compared with primary Supera stenting alone, suggested that the new generation DCBs could help achieve higher rates of primary patency and freedom from clinically-driven target lesion revascularization (CD-TLR), although there were no statistically significant differences due to limited sample size and a protocol requiring primary stenting.

The aim of this prospective, multicenter registry was to evaluate the safety and efficacy of the Legflow DCB for the treatment of femoropopliteal disease in an all-comers setting.

Materials and Methods

Study Design

A registry was established in January 2014 to collect data on all patients undergoing angioplasty using the Legflow DCB in the femoropopliteal segment at 4 European centers not involved in the RAPID randomized trial. Patients were treated following standard practice at each specific institution. The decision to use the Legflow was at the discretion of the operator. The follow-up protocol, which was approved by the institutional review board or ethics committee of each hospital, specified that patient examinations be conducted at hospital discharge, at 30 days, and at 6 and 12 months using duplex ultrasonography. Repeated angiography was performed when the peak systolic velocity ratio (PSVR) was between 2.4 to 5.0 (intermediate restenosis) in the presence of clinical symptoms or >5.0 (severe restenosis) regardless of symptom status and in cases of stent occlusion.[6]

Angioplasty Technique

In general, all patients were on aspirin (75–160 mg/d) and ticlopidine (250 mg twice daily) or clopidogrel (75 mg/d) prior to treatment. Procedures were performed percutaneously or surgically in the context of a hybrid procedure. Vascular access was achieved via the ipsilateral or contralateral common femoral artery with either a long or a short sheath to achieve adequate support. Unfractionated heparin (70–100 U/kg) was administered to maintain an activated coagulation time >250 seconds.

Once diagnostic angiography was completed, the wire was advanced though the target lesion into the distal popliteal artery. The target lesion was predilated at the operator's discretion. The Legflow balloon was sized 1:1 with the reference vessel diameter and of sufficient length to span the target lesion with 10-mm margins proximally and distally. If multiple balloons were required in lengthy lesions, 5-mm balloon overlap was allowed to obtain uniform drug delivery. The DEB was inflated at the nominal pressure for at least 90 seconds. Nitinol stent implantation was allowed in case of residual stenosis >30% or flow-limiting dissections. At the end of the procedure, access site hemostasis was achieved by manual compression or with the use of a closure device according to operator preference. After the procedure, ticlopidine or clopidogrel was prescribed for 30 days, whereas aspirin was continued for life.

Patient Population

From January 2014 to June 2016, 139 patients (mean age 67.1±10.8 years; 109 men) were enrolled in the registry. Patient and lesion characteristics are summarized in Table 1. Seventy-nine (56.8%) patients were claudicants, while 60 (43.2%) had CLI. More than half the lesions were de novo (80, 57.5%); a third (44, 31.6%) were occlusions. Mean lesion length (MLL) was 90±41.2 mm. Eighty (57.6%) patients were treated for de novo lesions (MLL 83.2±41.2 mm), 29 (20.9%) for postangioplasty restenosis (MLL 81.2±30.9 mm), and 30 (21.6%) for in-stent restenosis (MLL 117.0±39.5 mm).

Definitions and Outcome Measures

Technical success was defined as the ability to successfully perform balloon angioplasty and DCB postdilation with a residual stenosis <30% or <10% if bailout stent implantation was necessary.[7] Major adverse cardiovascular events

Table 1. Baseline Characteristics of the 139 Patients in the Registry.[a]

Age, y	67.1±10.8
Men	109 (78.4)
Diabetes	70 (50.3)
Hypertension	110 (79.1)
Hypercholesterolemia	68 (48.9)
Smoking history	71 (51.0)
Rutherford category	
2	20 (14.4)
3	59 (42.4)
4	41 (29.5)
5	19 (13.7)
Lesion characteristics	
De novo lesions	80 (57.5)
Restenoses	29 (20.8)
In-stent restenosis	30 (21.5)
Length, mm	90.0±41.2
De novo lesions	83.2±41.2
Native artery restenoses	81.2±30.9
In-stent restenoses	117.0±39.5
Occlusions	44 (31.6)

[a]Continuous data are presented as the means ± standard deviation; categorical data are given as the number (percentage).

(MACEs) included myocardial infarction, stroke, and cardiovascular death.[7] Major adverse limb events (MALEs) referred to the composite of acute limb ischemia, major amputation (not including forefoot or toe), or urgent revascularization (thrombolysis or other intervention for ischemia).[8] Clinical success was technical success without any in-hospital MACE or MALE.[6]

Arterial inflow revascularization involved any vessel proximal to the femoropopliteal lesion (ie, common femoral artery or the aortoiliac segment); arterial outflow revascularization involved the tibial arteries.[7] Concerning lesion length, focal lesions were <1 cm, short lesions ⩾1 and <5 cm, intermediate lesions ⩾5 and <15 cm, and long lesions ⩾15 cm.[7]

The primary outcome measure was freedom from binary restenosis as determined by a PSVR ⩾2.4 on duplex or >50% stenosis on digital subtraction angiography at 12 months. The secondary outcome was freedom from CD-TLR at 12 months.

Statistical Analysis

Continuous data are presented as the means ± standard deviation; categorical data are given as the number (percentage). Categorical variables were compared using the chi-squared or Fisher exact test as appropriate. The Student *t* test for independent samples was used to compare groups of continuous variables. The threshold of statistical significance was $p<0.05$. All data were analyzed using SPSS software (version 24.0 for Windows; IBM Corporation, Armonk, NY, USA).

Table 2. Characteristics of the 139 Procedures.[a]

Ipsilateral access	71 (51.1)
DCB diameter, mm	5.0±0.9
Cumulative DCB length, mm	113.5±48.0
Bailout stenting	39 (28.1)
De novo	30 (37.5)
Restenosis	7 (24.1)
In-stent restenosis	2 (6.7)
Associated revascularizations	
Inflow	23 (16.5)
Outflow	19 (13.6)
Inflow + outflow	5 (3.6)

Abbreviation: DCB, drug-coated balloon.
[a]Continuous data are presented as the means ± standard deviation; categorical data are given as the number (percentage).

Results

Technical success was achieved in all 139 treated patients. A simultaneous inflow revascularization procedure was necessary in 23 (16.5%) patients, while an outflow procedure was performed in 19 (13.6%); 5 (3.6%) patients had concomitant inflow and outflow revascularization.

Bailout stenting was necessary in 39 (28.1%) patients (Table 2), more frequently in de novo vs restenotic lesions (37.5% vs 11.8%, p=0.03). Among restenotic lesions, the need for bailout stenting was less frequent in the treatment of in-stent restenosis vs postangioplasty restenosis (6.0% vs 24.1%, p=0.06). Mean lesion length was comparable between lesions requiring bailout stenting and those that did not (90.5±39.9 vs 89.9±41.8 mm, $p>0.99$).

Six adverse events from intervention to discharge were reported (3 MALE and 3 MACE) in 5 (3.6%) patients; none was judged to be related to the use of DCB. An urgent TLR was necessary in 3 patients owing to SFA restenoses; all had CLI (p=0.047 vs claudicants). In 2, abrupt SFA occlusion occurred within 12 hours of the index procedure due to postdilation flow-limiting dissections that were not stented. The other case involved an acute stent occlusion. Similarly, 3 CLI patients experienced the only instances of in-hospital death (p=0.047 vs claudicants). One succumbed to sepsis following revascularization and planned minor amputation, another died as a consequence of an access site–related major bleeding, and the last one had hyperthermia after a surgical TLR.

During the period between treatment and the 12-month follow-up, 4 additional patients, 2 initially treated for claudication and 2 for CLI, died due to cardiac disease unrelated to the procedure. This left 132 patients available for 1-year follow-up. The primary outcome (freedom from restenosis)

was obtained in 107 (81.1%) patients. Freedom from CD-TLR was obtained in 110 (83.3%). Of the 25 late restenoses >50%, only 3 asymptomatic patients did not require TLR. Claudicant patients seemed to have a lower TLR rate compared to those with CLI (13.0% vs 21.8%, p=0.20); the difference was less evident if the 3 in-hospital urgent TLRs were excluded (13.0% vs 16.3%, p=0.63).

The type of lesion treated at the index procedure seemed to affect TLR rate. One-year TLR rates tended to be higher in restenotic lesions than de novo disease (22.2% vs 17.1%, p=0.55), but the opposite was observed for in-stent restenoses vs de novo lesions (10.3% vs 17.1%, p=0.38). As expected in evaluating de novo lesions, the longer the diseased segment, the higher the TLR rate (focal-short lesions 11.1% vs intermediate-long lesions 18.9%, p=0.09).

Discussion

Drug coating of medical devices has significantly improved long-term outcomes of angioplasty in the femoropopliteal arteries.[9] However, available devices and their coatings differ from one another. Despite the fact that large controlled trials have become the standard of clinical research for evaluating endovascular devices, these investigations do not necessarily represent the standard of care or diverse lesion morphologies in common clinical practice. The use of "real-world" registries could help provide a valuable assessment of safety and efficacy of a novel device in daily clinical practice.

The present study enrolled a patient population more typical of routine clinical practice than that of any comparable study.[10] The enrollees represented a wide range of lesion severity, including high percentages of patients with advanced disease (Rutherford category 4/5), intermediate length lesions, and restenotic lesions.

The paclitaxel dose of the study balloon was 3 $\mu g/mm^2$ balloon surface, close or identical to other coatings for which satisfactory efficacy has been shown in randomized trials.[11–14] In addition, this device is covered with a homogenous and stable surface coating containing nanoparticles of paclitaxel; these technological features contributed to satisfactory TLR rates in all clinical conditions.

Provisional stenting was performed in almost 30% of cases, which differs from the LEVANT studies (Lutonix Paclitaxel-Coated Balloon for the Prevention of Femoropopliteal Restenosis), in which patients with flow-limiting dissection or clinically significant residual stenosis were excluded from randomization.[11,12] The same is true for IN.PACT SFA (Randomized Trial of IN.PACT Admiral Drug-Eluting Balloon vs Standard Percutaneous Transluminal Angioplasty for the Treatment of Atherosclerotic Lesions in the Superficial Femoral Artery and/or Proximal Popliteal Artery)[13] and THUNDER (Local Taxane With Short Exposure for Reduction of Restenosis in Distal Arteries),[14] with about 10% stenting in each. A reason for the higher stenting rate may be related to the high percentage of complex lesions (ie, occlusions) treated in the present study.

The use of DCBs for the treatment of SFA in-stent restenosis has shown excellent results in terms of clinical benefit and primary patency over 2 years.[15] Similarly, in this multicenter registry, the use of the Legflow balloon for the treatment of in-stent restenosis was shown to be valuable at 12 months.

The 12-month safety and efficacy results indicate a substantial beneficial effect of the coating: as in other trials of paclitaxel-coated balloons in the SFA, there was no evidence of safety issues. These data are consistent with those reported in the RAPID trial of the Legflow plus primary Supera stenting vs primary Supera stenting alone.[5] In fact, the DCB group in the RAPID trial had higher rates of primary patency and freedom from CD-TLR, although there were no statistically significant differences.

One important clinical observation from this study was the high percentages of in-hospital MACE and MALE in the registry's CLI patients, which highlights the need to improve clinical and interventional care for CLI patients.

Limitations

The lack of a control group limits any definitive conclusions that can be made regarding the objective value of adding a DCB on top of the standard treatment. A further limitation of this study is that the angioplasty technique was not consistent throughout the 4 different institutions. Moreover, information related to TransAtlantic Inter-Society Consensus lesion classification and calcification scores were not collected in the registry. However, the relevance of the registry data in contemporary practice is supported by the observations that clinical or anatomical features did not influence the outcomes.

Conclusion

The reported data suggest that the use of a new generation paclitaxel-coated balloon represents a safe and effective therapeutic strategy for the endovascular treatment of femoropopliteal obstructions in different clinical and anatomical settings. These data will need to be confirmed with longer-term follow-up and in randomized controlled trials.

Declaration of Conflicting Interests

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Residual ADDED index to risk-stratify STEMI patients after PCI

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Running title: Residual Added Index in STEMI patients

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Key words: STEMI, ADDED Index, Syntax Score, Multivessel Disease

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Abstract

Background. Multivessel disease (MVD) is common (in approximately 50%) in patients with STEMI. MVD significantly impairs short and long-term outcome. Functional scores, such as the FFR-guided SYNTAX score (SS) predicted clinical outcome better than the classic SS in patients with MVD undergoing primary percutaneous coronary intervention (pPCI). We have recently validated the "Angiography-DeriveD hEmoDynamic index" (ADDED index), able to predict the functional relevance of coronary artery stenosis.

Methods. We retrospectively included 596 STEMI patients in whom pPCI was successful. Both rAI and residual SS (rSS) were calculated. All patients were divided in rAI tertiles and rSS tertiles, patients without residual coronary artery stenosis served as control group. Primary end points were: 1) major adverse cardiac events (MACE), composite of overall death, myocardial infarction, clinically driven revascularizations; 2) non-target vessel oriented adverse clinical events (VOCE), composite of overall death, deferred non-target vessel related myocardial infarction and deferred non-target vessel related clinically driven revascularizations.

Results. Follow-up was obtained in 98% of patients at a median of 24 months (14–36 months). At multivariate analysis, MACE rate was significantly higher in the third tertile of both rAI and rSS. The rate of VOCE was equally significantly higher in the

third tertile of both rAI and rSS. Logistic regression analysis showed a significant increase in MACE and VOCE risk for every unitary increase in rAI such as in rSS.

Conclusions. The rAI represents a simple prognostic tool for patients with residual coronary artery stenosis after pPCI, allowing prediction of 2-years MACE and VOCE as well as a validated index such as rSS.

Introduction

Multivessel coronary artery disease (MVD) occurs in about 50% of the patients undergoing primary percutaneous coronary intervention (pPCI) and affects both in-hospital and long-term clinical outcomes. (1,2) While it is obviously recommended to treat the infarct-related artery (IRA), evidence supporting revascularization of residual "significant" coronary artery stenosis is conflicting. (3) Some recent randomized trials showed an improved clinical outcome in patients undergoing complete revascularization as compared with patients in whom pPCI was only performed. (4-6) However, the timing and the real meaning of "complete" revascularization (i.e., angio vs ischemia and/or FFR guided) remain controversial, so incomplete coronary revascularization after pPCI remains. (7)

The residual SYNTAX Score (rSS) has been previously suggested to quantify the degree of residual stenosis after PCI and it has also been showed to predict outcomes at 1 year. (8) However, the rSS reflects only angiographic stenosis complexity regardless of its related ischemic potential. A fractional flow reserve (FFR)-guided SYNTAX Score (SS), named "functional SS", predicted clinical outcome better than the classic SS in patients with MVD undergoing PCI in the cohort of the FAME study. (9,10) The ADDED index (AI), namely the ratio between the Duke Jeopardy Score (DJS) and the minimal lumen diameter (MLD) of the target coronary artery stenosis as assessed at the quantitative coronary angiography (QCA), showed high accuracy to predict FFR and it might be used to detect functionally significant coronary artery stenosis. An AI lower than 2.23 indicates functionally non-significant coronary artery stenosis with an accuracy of 86%, while an AI higher than 2.9 has a strong predictive value for a

significantly reduced FFR. (11) Recently, we demonstrated the improved prognostic value of residual AI (rAI), in comparison with visually estimation of residual coronary stenosis in patients presenting with STEMI and MVD after successful PCI of the culprit lesion. (12)

The aim of the present study was to investigate, in patients with STEMI undergoing successful primary percutaneous coronary intervention (pPCI), the prognostic value of rAI Index (rAI) as compared with rSS.

Methods

Patient population

All consecutive patients presenting with STEMI who referred to our department for primary, rescue or elective PCI between January 2011 and December 2015 were included. Diagnosis of STEMI and clinical/interventional management was made according to the current guidelines. (3) PCI was performed according to the conventional strategies and operators' routine practice and could include direct stenting vs predilation, aspiration thrombectomy, heparin and/or glycoprotein IIb/IIIa inhibitor administration. Antiplatelet therapy included aspirin and a P2Y12 inhibitor (clopidogrel, ticagrelor and prasugrel). Patients with previous coronary artery bypass grafts (CABG), chronic coronary artery occlusions (CTO), undergoing not successful PCI of the culprit coronary artery stenosis, or patients who did not survive after the index procedure were excluded. In patients with MVD, the decision to perform non-culprit coronary artery PCI either during the index procedure or in a staged session was left to the judgement of the interventional cardiologist. Staged PCI

was defined as revascularization of at least one non-culprit lesion during the index admission or planned in the following 30 days.

Data collection and follow-up

The study conformed to the Declaration of Helsinki on human research, and all patients gave informed consent. Demographic, clinical, echocardiographic, angiographic and laboratory data at admission were collected and recorded in a computerized database, in accordance with our department's protocol for patients with STEMI undergoing PCI. Digitally archived diagnostic and procedural angiograms were reviewed by two interventional cardiologists blinded to clinical outcomes. Intermediate coronary artery stenosis was defined on the basis of visual estimation as those determining a reduction of vessel diameter comprised between 30% and 70%. (13) Localization on the coronary tree and quantitative assessment of stenosis severity at coronary angiography was performed, offline, independently by 2 expert interventional cardiologists, blinded to clinical and hemodynamic data. Two-dimensional QCA was performed offline using the cardiovascular angiography analysis system (Pie Medical Imaging, Maastricht, the Netherlands). Automated distance calibration was used to determine pixel size. All analyses were performed during the ECG-gated end-diastolic frame. Reference vessel diameter (RVD), lesion length (LL), minimal lumen diameter (MLD), and percentage diameter stenosis (DS) were calculated. rSS and rAI were calculated based on the coronary angiograms at the end of primary and staged revascularization. rSS was obtained as previously described. (1) For the calculation of rAI, the amount of perfused myocardium

subtended by the target stenosis was assessed using the Duke Jeopardy Score (DJS). (11,14) Briefly, the coronary tree was divided into 6 segments: the LAD, diagonal branches of the LAD, septal perforating branches, the circumflex coronary artery, obtuse marginal branches, and the posterior descending coronary artery. Two points were assigned to each of these segments. All segments distal to the index stenosis were considered to be at risk. The maximum possible score is 12 for all myocardial mass and, for example, 6 for the proximal LAD. The AI for a single vessel was defined as the ratio between the DJS and the MLD as respectively assessed at angiography and QCA. (11) All patients were divided in rAI tertiles (first <1.79 [n=94], second from 1.79 to 3.49 [n=93], and third >3.49 [n=97]) and rSS tertiles (first <3.00 [n=83], second from 3.00 to 6.00 [n=107], and third >6.00 [n=94]), patients without residual coronary artery stenosis served as control group (n=321). Clinical follow-up was performed using hospital records and telephone interviews, and it was conducted up to 2 years. All events were classified and adjudicated by a physician who was unaware of the treatment assignment and of the angiographic details of the lesions. Primary end points were: 1) major adverse cardiac and events (MACEs), defined as the composite end point of all-cause death, nonfatal MI, non-planned ischemia-driven revascularization (both urgent and non-urgent); 2) non-target vessel-oriented clinical events (VOCE), composite of overall death, deferred non-target vessel related myocardial infarction and deferred non-target vessel related ischemia-driven revascularizations. Staged procedures were not considered as events. All events were classified and adjudicated by a physician who was unaware of the study group and of the angiographic details of the lesions.

Statistical analysis

Continuous variables are presented as mean ± standard deviation (SD) or median with interquartile range (IQR) as appropriate. Normal distribution was assessed by the Kolmogorov-Smirnov test. Categorical variables are reported as frequencies and percentages. Comparisons between categorical variables were evaluated using Pearson $\chi 2$ test or Fisher test. Continuous variables were compared using one-way ANOVA and relative post-hoc analysis. Two-years cumulative rates of MACE and VOCE were estimated by Kaplan-Meier method and Cox regression. Cox regression analysis was adjusted by propensity score (PS) evaluation and used to compare clinical and procedural variables between the groups. Relative risk for unitary increase of rSS and rAI was estimated using logistic regression analysis. Data were analyzed with SPSS, version 25.0, software (SPSS Inc., Chicago, Illinois) and GraphPad Prism 7.00 (GraphPad Software Inc., La Jolla, California). A probability value of <0.05 was considered significant.

Results

Clinical and procedural characteristics of included patients

A total of 596 patients were included: 314 in the Control group, 93 in the first rAI tertile (≤ 1.79), 92 in the second rASI tertile (between 1.79 and 3.49), 97 in the third rAI group (≥ 3.49), 81 in the first rSS tertile (≤ 3), 90 in the second rSS tertile (between 3 and 6), and 111 in the third rAI tertile (≥ 6). When compared with control group, patients in the third rAI tertile were significantly older, were more frequently affected

by arterial hypertension, hyperlipidemia, diabetes mellitus, peripheral arterial disease and/or atrial fibrillation **(Table 1A)**. Patients in the third rSS, in comparison with control group, were significantly older, were more frequently affected by arterial hypertension, hyperlipidemia, diabetes mellitus, peripheral arterial disease, atrial fibrillation, and/or chronic kidney disease **(Table 1B)**. No statistically relevant difference was observed for gender, ejection fraction, smoking, previous MI, previous PCI and family history of CAD **(Table 1A and Table 1B)**.

Concerning procedural data, both in rAI and rSS groups no statistically relevant di differences in terms of strategy of revascularization (pPCI, rescue PCI or elective PCI after successful thrombolysis) was observed in comparison with control group. On the other hand, differences were observed, both in the third rAI and rSS tertiles in comparison with control group, in terms of number of coronary lesions, culprit vessel treated with PCI and medium diameter of implanted stents **(Table 2A and Table 2B)**.

Clinical outcome

Follow-up was obtained in 98% of patients at a median of 24 months (IQR: 14–36 months). As described in **Table 3A and Table 3B,** at the Propensity Score adjusted Cox's analysis, MACE rate was significantly higher in the third tertile of rAI (26%) in comparison with control group ((8%, HR:3.00 (1.63-5.53)) and with first tertile group (7%, HR:3.24 (1.19-8.83)), while incidence of VOCE was significantly increased only in the third terile group (21%) in comparison with control group (4%, HR:4.91 (2.28-10.56)).

Conversely, when patients were stratified according to rSS, MACE incidence resulted significanlty increased only in the third tertile group (25%) in comparison with patients in the first tertile group (5%, HR:3.39 (1.01-11.34)).

At Kaplan Meier analysis, we observed, after stratifying patients in rAI tertiles, we observed a progressive, significant reduction in MACE and VOCE free survival after 24 months follow-up, particularly in the third tertile rAI group. At the same time, after stratifying patients in rSS tertiles, we observed a significant reduction in MACE and VOCE free survival after 24 months follow-up with increased rSS, particularly in the third tertile rSS group **(Figure 3 and Figure 4).**

Moreover, when performing a logistic regression analysis, we observed a significant increase in MACE and VOCE risk for every unitary increase in rAI (respectively, 32% [HR:1.32(1.17-1.49),P<0,001] and 40% [HR:1.40(1.23-1.59),p<0.001]; at the same time, we observed a significant increase in MACE and VOCE risk for every unitary increase in rSS (respectively, 19% [HR:1.19 (1.15-1.34), p<0,001] and 25% [1.25(1.15-1.34), p<0,001]. (**Figure 5-8).**

Discussion

This retrospective analysis confirms the potential, for rAI, to identify patients at higher cardiovascular risk after treatment of STEMI. In our precedent publication, we showed that rAI performed a better prognostic stratification when compared with visually estimation of residual coronary artery stenosis after pPCI. (12) In this paper,

we demonstrate that rAI has a potential for prognostic assessment resulting to be comparable to rSS.

SS is a worldwide index to stratify complexity of coronary artery disease on the basis of an anatomical assessment. In the first half of 2010's decade, the calculation of rSS after percutaneous coronary artery revascularization, i.e. the SS deriving from significant coronary artery stenosis left untreated, has been defined as an efficient tool to predict cardiovascular prognosis. In the cohort of the prospective ACUITY (Acute Catheterization and Urgent Intervention Triage Strategy) trial, (15) a subanalysis enrolling 2,686 patients with acute coronary syndrome stratified patients according to rSS. Patients with rSS>0 had 30-day and 1-year rates of ischemic events higher than those with complete revascularization, especially those with high rSS (> 8). (16) Similarly, in the randomized PCI cohort of the SYNTAX Trial (n=903), (17) patients without residual coronry artery stenosis and patients with rSS ≤8 had a comparable 5-year mortality, while a rSS > 8 was associated with all-cause mortality at 5-years of about 35%. (18) It should be noted that calculation of rAI is consistently faster and simple than rSS, so the demonstrated equivalence for prognostic stratification power makes the first one relevant and appealing. To be noted, in the cohort of the FAME trial, (19) patients with a significant rSS on the basis of the presence of residual stenosis without physiological relevance (i.e., a fractional flow reserve > 0.80) do not have an impaired prognosis. (20) Notably, rAI has the potential to discriminate for physiological relevance of coronary artery stenosis, differently from rSS, being AI a predictor for invasive FFR value, (11) which avoid, at the same time, concerns related to invasive assessment (adenosine use, wire manipulation,

hemodynamic tolerance) particularly in the setting of acute myocardial infarction/pPCI.

The correct management of residual coronary artery stenosis after pPCI remains a residual field of uncertainness. (7) A significant number of trials (such as the the CVLPRIT, the DANAMI-3-PRIMULTI and the COMPARE ACUTE) (4-6) have clearly shown that complete revascularization should become a dogma for these patients, at higher risk of future cardiovascular events, but the correct management remains controversial. Moreover, the prognostic improvement is mostly related to prevention of successive myocardial infarction and clinically driven revascularizations, so a correct definition of the lesions to be treated appears mandatory. Issues remain regarding the correct timing and guiding criteria for interventions, with differences also in the cited randomized trials about definition of residual significant coronary artery stenosis on an angiographic and/or a functional basis. (7) To avoid the rely on subjective visual estimation, functional evaluation for the presence of inducible ischemia by invasive pressure wire based devices with the classic FFR or with newly validated resting index such as iFR has been proposed and seems to be feasible and effective, even with some concern when performed in the hyperacute phase, as written above. However, the results of a recent trial (the FLOWER-MI) are concerning. (21) In this study 586 patients with STEMI and multivessel disease underwent FFR-guided revascularization and 577 other patients in the same clinical context underwent angiography-guided revascularization. (21) During follow-up, a primary outcome event (a composite of death from any cause, nonfatal myocardial infarction, or unplanned hospitalization leading to urgent revascularization at 1 year) occurred

in 32 patients (5.5%) in the FFR-guided group and in 24 of 577 patients (4.2%) in the angiography-guided group (hazard ratio, 1.32; 95% confidence interval, 0.78 to 2.23; P=0.31) FFR-guided strategy did not show a significant benefit over the angiography-guided strategy with respect to the risk of death, myocardial infarction, or urgent revascularization at 1 year. (21) While, given the wide confidence intervals for the estimate of effect, these findings do not allow for a conclusive interpretation, it is possible that other factors than the ischemic burden of the lesion have to been taken into account for an adequate and correct decision making. One of them could be the "prognostic" relevance of the lesion. In our precedent analysis we validated the rAI as an independent prognostic factor after STEMI. (12) In this study, we confirm our precedent observation by observing equipollence between rAI and a universally validated prognostic index such as rSS. So, rAI could be used for prognostication of patients at higher risk of future cardiovascular events, which could derive benefit from treatment of intermediate stenosis. Use of an easy-to-calculate angiographic index favors the avoidance of invasive manipulation with pressure wire, which could be desirable in some setting, reducing the risk of performing clinically unnecessary coronary interventions.

This study has several limitations, first of all related to its nature of retrospective analysis: that is, events underreporting, low event rate, especially for death, bias related to the operator's decision as to the revascularization strategy to be adopted, and many other potential confounding factors. In particular, the decision to perform complete coronary revascularization might have been influenced by several clinical and procedural features; we tried to minimize their impact by performing a

propensity score adjusted Cox regression analysis to assess the clinical outcome. FFR was not applied to guide revascularization of non-culprit intermediate coronary artery stenosis and non-invasive functional testing was available only in a few patients but we are unable to evaluate whether they have been used for PCI guidance. Even though most of the staged procedures (95%) were performed during the index hospitalization, we cannot exclude that some deferred non-culprit PCI, particularly those occurred within 6 months from discharge, might have been originally planned later than 30 days for several reasons that we cannot account due to the retrospective nature of the present study. MLD and visually estimated DS was derived by the angiography performed for pPCI and, in such adrenergic context, this might have leaded to an overestimation of the stenosis severity.

Conclusions

rAI represents a simple tool for prognostic evaluation of patients with residual coronary artery stenosis after pPCI, allowing prediction of 2-years MACE and VOCE as well as rSS. Identifying patients at higher risk of future cardiovascular events would be of paramount relevance to define an appropriate treatment for them, not only regarding the need for complete revascularization, but also in terms of optimization of drug therapy, pharmacological adherence, lifestyle modification and frequency of follow-up assessments.

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Table 1A - Clinical Characteristics

	No Residual Stenosis	Residual Stenosis			
		Residual Added Index 1T	Residual Added Index 2T	Residual Added Index 3T	p value
n	314	93	92	97	
Age	59 ± 12	61 ± 11	63 ± 12*	66 ± 13*	<0.01
Male Gender	244 (78)	71 (76)	72 (78)	73 (75)	0.95
EF (%)	43 ± 7	43 ± 6	46 ± 7	42 ± 8	0.07
DM	58 (18)	16 (17)	17 (18)	33 (34)*"^	0.006
HTN	169 (54)	51 (55)	62 (67)*	66 (68)*	0.02
HPL	112 (36)	31 (33)	41 (45)	50 (51)*"	0.02
Smoker	131 (42)	32 (34)	37 (40)	33 (34)	0.41
Previuos MI	23 (7)	10 (11)	6 (6)	12 (12)	0.32
Previuos PCI	19 (6)	9 (10)	6 (6)	7 (7)	0.68
PAD	1 (0)	1 (1)	4 (4)*	4 (4)*	0.01
CKD	7 (2)	1 (1)	2 (2)	6 (6)	0.12
Familiar hystory	86 (27)	19 (20)	33 (36)"	28 (29)	0.13
Atrial Fibrillation	2 (1)	1 (1)	0 (0)	4 (4)*	0.03

*: p<0.05 vs CTRL, ": p<0.05 vs AI1T, ^: p<0.05 vs AI2T

Table 1B - Clinical Characteristics

	No Residual Stenosis	Residual Stenosis			
		Residual Syntax Score 1T	Residual Syntax Score 2T	Residual Syntax Score 3T	p value
n	314	81	90	111	
Age	59 ± 12	62 ± 12	62 ± 11	65 ± 12*	<0.01
Male Gender	245 (78)	61 (75)	69 (77)	85 (77)	0.97
EF (%)	43 ± 7	43 ± 6	45 ± 6	43 ± 8	0.4
DM	58 (18)	14 (17)	17 (19)	35 (32)*"	0.03
HTN	169 (54)	39 (48)	61 (68)*"	79 (72)*"	0.001
HPL	113 (36)	28 (35)	36(40)	57 (52)*"	0.02
Smoker	131 (42)	23 (28)*	36 (40)	43 (39)	0.19
Previuos MI	23 (7)	6 (7)	9 (10)	13 (12)	0.47
Previuos PCI	19 (6)	6 (7)	8 (9)	8 (7)	0.80
PAD	1 (0)	1 (1)	0 (0)	8 (7)*^	<0.01
CKD	7 (2)	0 (0)	2 (2)	7 (6)"	0.04
Familiar hystory	86 (27)	16 (20)	29 (32)	35 (32)	0.22
Atrial Fibrillation	2 (1)	1 (1)	0 (0)	4 (4)*	0.05

*: p<0.05 vs CTRL, ": p<0.05 vs AI1T, ^: p<0.05 vs AI2T

Table 2A - Procedural Characteristics

	No Residual Stenosis	Residual Stenosis			
		Residual Added Index 1T	Residual Added Index 2T	Residual Added Index 3T	p value
n	314	93	92	97	
Revascularization Strategy					0.54
Elective PCI	61 (19)	17 (18)	15 (16)	17 (17)	
Rescue PCI	58 (18)	10 (11)	17 (18)	13 (13)	
Primary PCI	195 (62)	66 (71)	60 (65)	67 (69)	
Number of Diseased Vessels					<0.01
1	222 (71)	0 (0)*	0 (0)*"	0 (0)*"^	
2	82 (26)	79 (85)	55 (60)	34 (35)	
3	10 (3)	14 (15)	37 (40)	63 (65)	
MVD	92 (29)	93 (100)*	92 (100)*	97 (100)*	<0.01
Target Vessel PCI					0.03
LAD	173 (55)	54 (58)	35 (38)*"	45 (46)	
LCX	32 (10)	12 (13)	18 (20)	16 (16)	
RCA	109 (35)	27 (29)	39 (42)	36(37)	
Stent Diameter	3.1 ± 0.4	3.0 ± 0.4	3.0 ± 0.5*	3.0 ± 0.4	0.01
Stent Lenght	26 ± 14.0	29 ± 16	31 ± 16*	27 ± 15	0.05
Stent Type					0.24
Bare Metal Stent	28 (9)	4 (4)	7 (8)	12 (13)	
Drug Eluting Stent	279 (91)	85 (93)	84 (91)	81 (85)	
Bioresorbable Scaffold	1 (0)	2 (2)	1 (1)	2 (2)	
PCI non TV	92 (29)	20 (21)	17 (18)*	24 (25)	0.14
Mutivessel PCI	33 (36)	5 (25)	5 (29)	7 (29)	0.76
Residual Stenosis with Added Index > 2.23	-	0(0)	44 (48)*"	95(98)*"^	<0.01

*: p<0.05 vs CTRL, ": p<0.05 vs AI1T, ^: p<0.05 vs AI2T

Table 2B - Procedural Characteristics

	No Residual Stenosis	Residual Stenosis			
		Residual Syntax Score 1T	Residual Syntax Score 2T	Residual Syntax Score 3T	p value
n	314	81	90	111	
Revascularization Strategy					0.71
Elective PCI	61 (19)	13 (16)	15 (17)	21 (19)	
Rescue PCI	58 (18)	10 (12)	15 (17)	15 (14)	
Primary PCI	196 (62)	58 (72)	60 (67)	74 (67)	
Number of Diseased Vessels					<0.01
1	222 (70)	0 (0)	0 (0)*"	0 (0)*"^	
2	82 (26)	66 (81)*	57 (63)	45 (41)	
3	11 (3)	15 (18)	33 (37)	65 (59)	
MVD	93 (29)	81 (100)*	90 (100)*	110 (100)*	<0.01
Target Vessel PCI					<0.01
LAD	174 (55)	58 (72)*	39 (43)"	36 (33)*"	
LCX	32 (10)	8 (10)	16 (18)	22 (20)	
RCA	109 (35)	15 (18)	35 (39)	52 (47)	0.04
Stent Diameter	3.1 ± 0.4	2.9 ± 0.4*	3.0 ± 0.5	3.1 ± 0.4	0.002
Stent Lenght	26.5 ± 14.0	28.3 ± 16	31.0 ± 16.8	28 ± 15	0.08
Stent Type					0.03
Bare Metal Stent	28 (9)	4 (5)	5 (6)	14 (13)	
Drug Eluting Stent	280 (91)	73 (91)	84 (94)	92 (85)	
Bioresorbable Scaffold	1 (0)	3 (4)*	0 (0)	2 (2)	
PCI non TV	93 (29)	20 (25)	20 (22)	20 (18)*	0.10
Mutivessel PCI	33 (35)	5 (25)	6 (30)	6 (30)	0.80
Residual Stenosis with Added Index > 2.23	-	11(14)*	35 (39)*"	92 (84)*"^	<0.01

*: p<0.05 vs CTRL, ": p<0.05 vs AI1T, ^: p<0.05 vs AI2T

Table 3A - Clinical events at 24 moths follow up

	No Residual Stenosis n 309	Residual Added Index 91	Unadjusted HR (95%CI)	p value	PS Adjusted HR (95%CI)*	p value	Residual Added Index 92	Unadjusted HR (95%CI)	p value	PS Adjusted HR (95%CI)*	p value	Residual Added Index 3T 93	Unadjusted HR (95%CI)	p value	PS Adjusted HR (95%CI)*	p value
	11 (4)	5 (5)					2 (2)					6 (6)				
Overall Death	Reference		1.58 (0.55-4.56)	0.39				0.63 (0.14-2.84)	0.55				1.90 (0.70-5.13)	0.21		
		Reference						0.39 (0.08-2.02)	0.26				1.21 (0.37-3.97)	0.75		
							Reference						3.07 (0.62-15.23)	0.17		
	7 (2)	1(1)					4 (4)					6 (6)				
Acute Coronary Syndrome	Reference		0.49 (0.06-3.95)	0.50				1.94 (0.57-6.65)	0.29				3.01 (1.01-8.96)	0.048		
		Reference						4.04 (0.45-36.2)	0.21				6.14 (0.74-51.04)	0.09		
							Reference						1.57 (0.44-5.57)	0.48		
	0 (0)	0 (0)					3 (3)					3 (3)				
Deferred NonTV-Related Acute Coronary Syndrome	Reference							-	-				-	-		
		Reference											1.05 (0.21-5.19)	0.95		
							Reference									
	12 (4)	2 (2)					10 (11)					16(17)				
Overall Revascularization	Reference		0.57 (0.13-2.57)	0.47				2.87 (1.24-6.64)	0.014				4.89 (2.31-10.35)	<0.001		
		Reference						5.01 (1.1-22.88)	0.004				8.41 (1.93-36.59)	0.005		
							Reference						1.74 (0.79-3.83)	0.17		
	0 (0)	1 (1)					10 (11)					14 (15)				
Non-Target Vessel Revascularization	Reference							-	-				-	-		
		Reference						10.04 (1.28-78.46)	0.03							
							Reference						1.52 (0.67-3.42)	0.31		
	12 (4)	6 (7)					12 (13)					20 (21)				
Non-Target Vessel Oriented Major Adverse Cardiovasacular Event	Reference		1.76 (0.66-4.69)	0.26	1.87(0.55-6.39)	0.32		3.58 (1.61-7.98)	0.002	5.30(0.89-31.48)	0.07		6.09 (2.97-12.46)	<0.001	4.91(2.28-10.56)	<0.001
		Reference						1.98 (0.74-5.29)	0.17	1.69(0.59-4.83)	0.32		3.47 (1.39-8.63)	0.01	2.61(0.92-7.38)	0.07
							Reference						1.77 (0.87-3.63)	0.12	1.63(0.67-3.47)	0.20
	24 (8)	6 (7)					13 (14)					24 (26)				
Major Adverse Cardiovasacular Event	Reference		0.87 (0.35 - 2.12)	0.76	0.76(0.27-2.12)	0.60		1.89 (0.96-3.71)	0.06	3.27(0.72-14.84)	0.12		3.70 (2.10-6.52)	<0.001	3.00(1.63-5.53)	<0.01
		Reference						2.15 (0.82-5.67)	0.12	2.00(0.71-5.63)	0.19		4.24 (1.73-10.38)	0.002	3.24(1.19-8.83)	0.02
							Reference						2.00 (1.02-3.93)	0.04	1.84(0.91-3.73)	0.09

Table 3B - Clinical events at 24 moths follow up

	No Residual Stenosis n 308	Residual Syntax Score 78	Unadjusted HR (95%CI)	p value	PS Adjusted HR (95%CI)*	p value	Residual Syntax score 90	Unadjusted HR (95%CI)	p value	PS Adjusted HR (95%CI)*	p value	Residual Syntax score 3T 108	Unadjusted HR (95%CI)	p value	PS Adjusted HR (95%CI)*	p value
	11 (4)	2(3)					6(7)					5(5)				
Overall Death	Reference		0.76(0.17-3.42)	0.72				1.94(0.72-5.24)	0.19				1.35(0.47-3.88)	0.58		
		Reference						2.57(0.52-12.73)	0.25				1.83(0.35-9.44)	0.47		
							Reference						0.71(0.22-2.33)	0.57		
	7 (2)	2(3)					2(2)					7(6)				
Acute Coronary Syndrome	Reference		1.14(0.24-5.51)	0.87				0.99(0.21-4.79)	0.99				3.03(1.06-8.65)	0.04		
		Reference						0.86(0.12-6.13)	0.88				2.57(0.53-12.38)	0.24		
							Reference						3.03(0.63-14.61)	0.17		
	0 (0)	0 (0)					2(2)					4(4)				
Deferred NonTV-Related Acute Coronary Syndrome	Reference							57.97(0.001-5200000	0.48				50 (0.01-740000)	0.35		
		Reference											1.73(0.32-9.47)	0.52		
							Reference									
	12 (4)	2(3)					6(7)					20(19)				
Overall Revascularization	Reference		0.68(0.15-3.03)	0.61				1.67(0.66-4.71)	0.25				5.25(2.67-10.75)	<0.001		
		Reference						2.57(0.52-12.76)	0.25				7.7(1.80-33)	0.006		
							Reference						2.94(1.18-7.31)	0.02		
	0 (0)	1 (1)					6(7)					18(17)				
Non-Target Vessel Revascularization	Reference							5.14(0.62-4.72)	0.13				13.94(1.86-104)	0.01		
		Reference											2.64(1.05-6.66)	0.04		
							Reference									
	12 (4)	3(4)					12(13)					23(21)				
Non-Target Vessel Oriented Major Adverse Cardiovasacular Event	Reference		1.05(0.29-3.71)	0.94	1.14(0.25-5.23)	0.87		3.62(1.63-8.07)	0.002	3.56(1.27-10.00)	0.02		6.04(3.00-12.14)	<0.001	2.49(0.37-16.73)	0.35
		Reference						3.44(0.97-12.19)	0.06	2.91(0.76-11.15)	0.12		5.82(1.75-19.39)	0.004	3.72(0.94-14.73)	0.06
							Reference						1.67(0.83-3.64)	0.15	1.53(0.74-3.18)	0.25
	24 (8)	4(5)					12(13)					27(25)				
Major Adverse Cardiovasacular Event	Reference		0.69(0.24-2.00)	0.49	0.58(0.18-1.94)	0.38		1.78(0.89-3.56)	0.10	1.56(0.65-3.77)	0.32		3.57(2.06-6.18)	<0.001	1.93(0.42-8.84)	0.40
		Reference						2.57(0.83-7.97)	0.10	2.33(0.69-7.82)	0.17		5.19(1.81-14.83)	0.002	3.39(1.01-11.34)	0.048
							Reference						2.00(1.01-3.95)	0.046	1.88(0.93-3.82)	0.08

Figure 1. *Kaplan-Meier curve: events (MACE) free survival analysis after 24 months-follow up into rAI tertiles.*

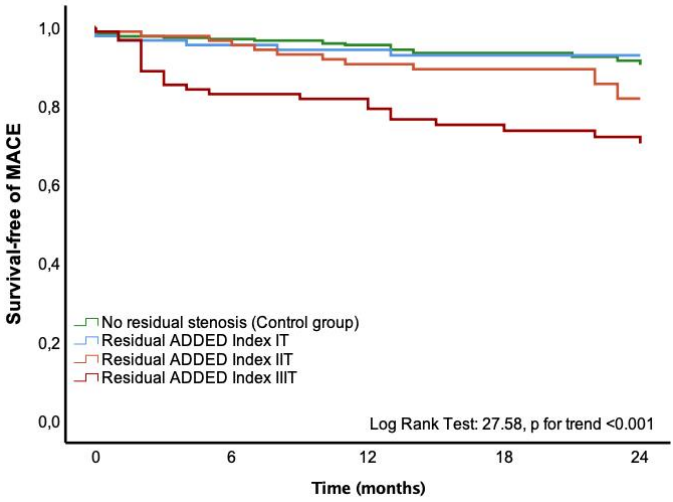


Figure 2. *Kaplan-Meier curve: events (VOCE) free survival analysis after 24 months-follow up into rAI tertiles.*

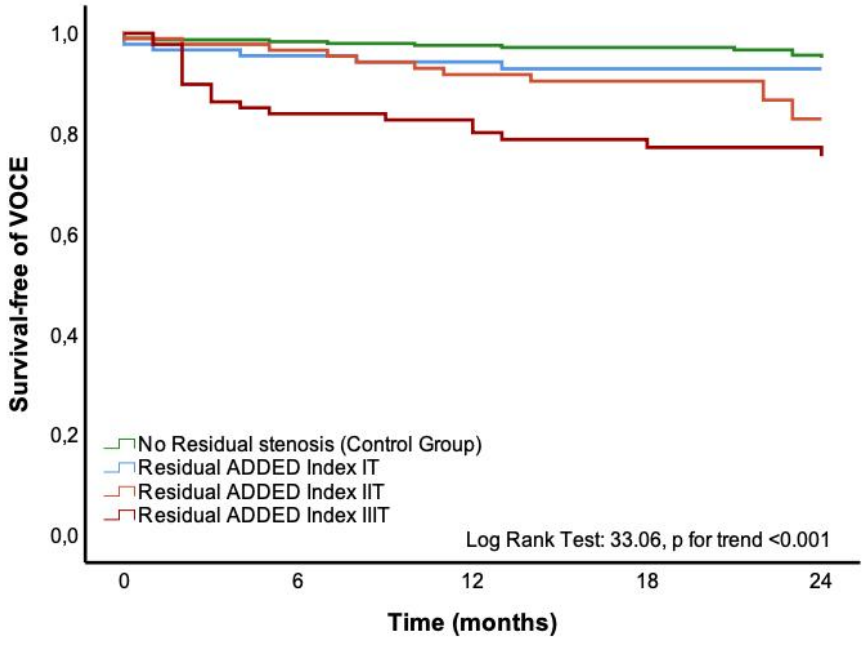


Figure 3. *Kaplan-Meier curve: events (MACE) free survival analysis after 24 months-follow up into rSS tertiles.*

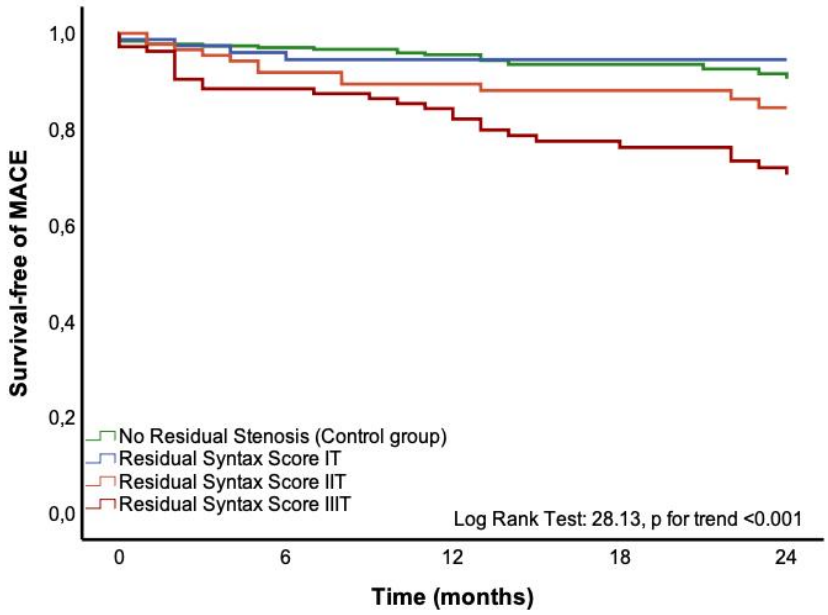


Figure 4. *Kaplan-Meier curve: events (VOCE) free survival analysis after 24 months-follow up into rSS tertiles.*

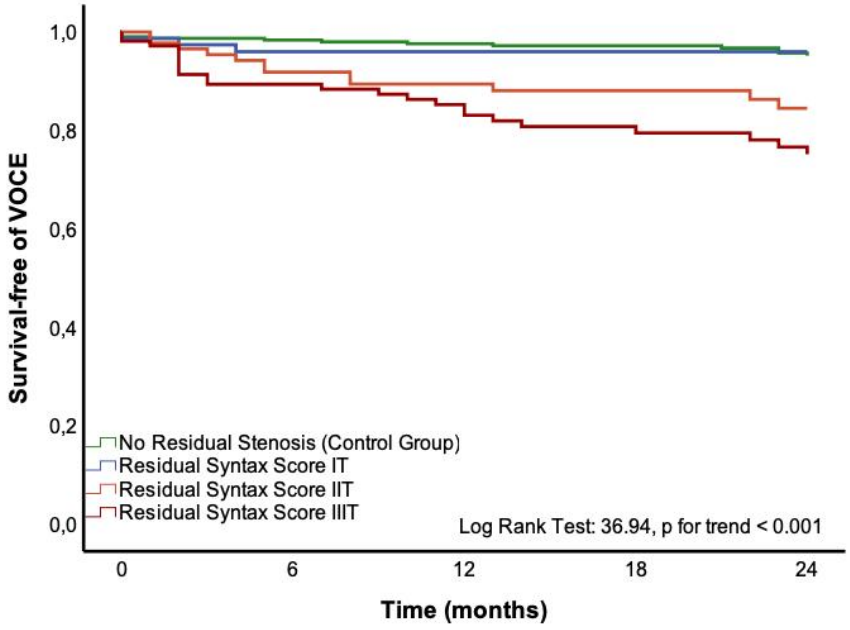


Figure 5. *Logistic regression model: relation between MACE risk and unitary increase in rAI value*

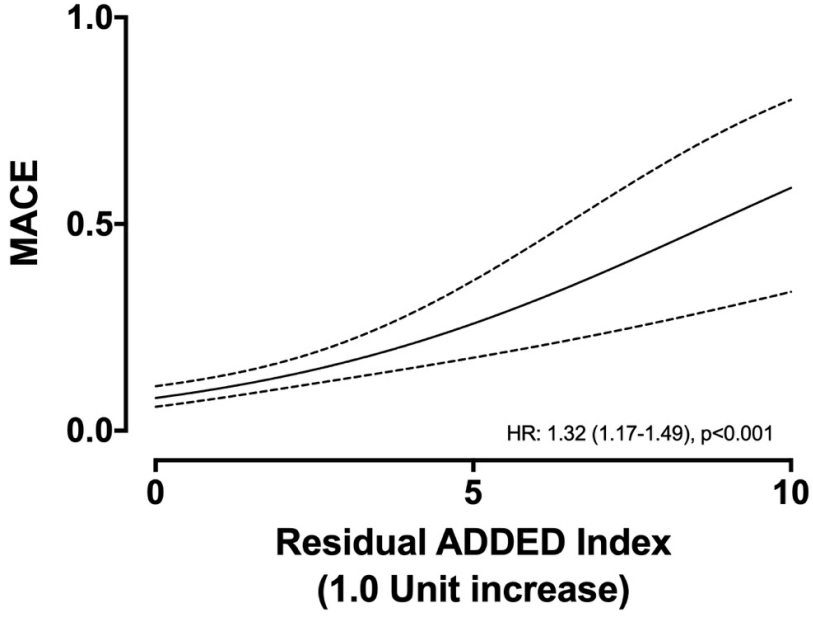


Figure 6. *Logistic regression model: relation between VOCE risk and unitary increase in rAI value*

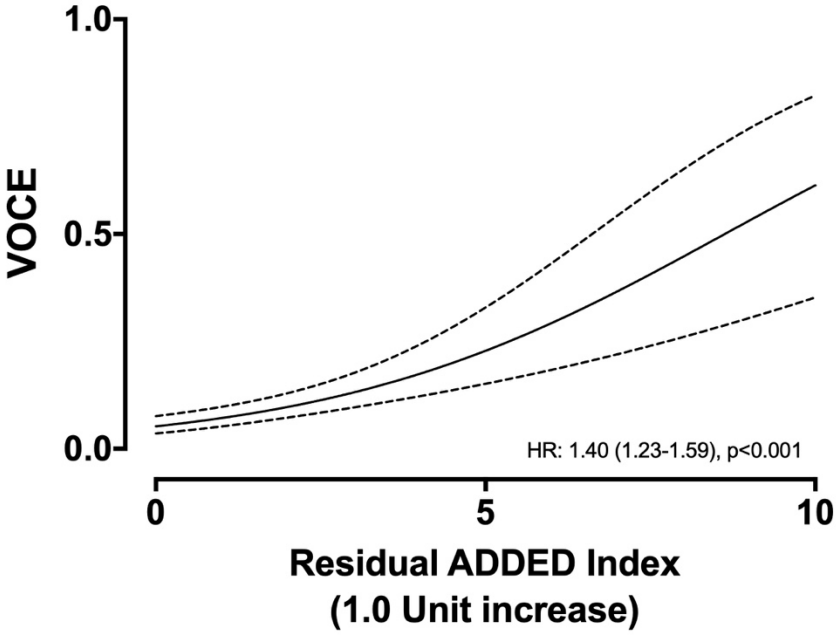


Figure 7. *Logistic regression model: relation between MACE risk and unitary increase in rSS value*

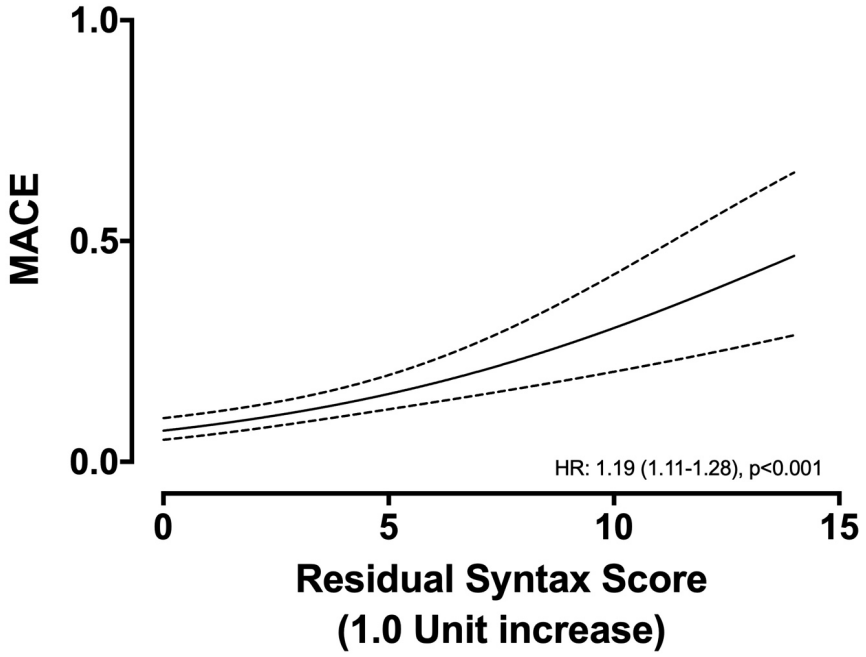
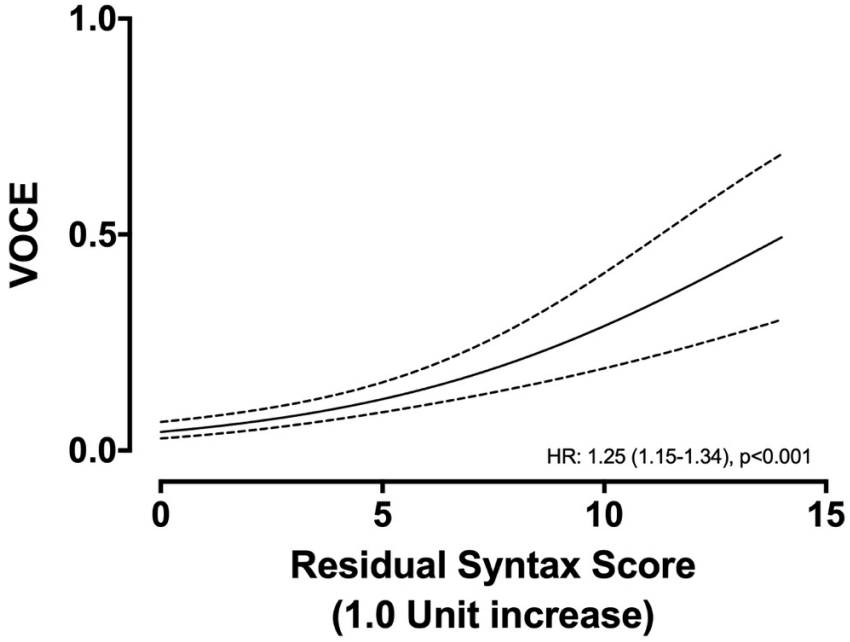


Figure 8. *Logistic regression model: relation between VOCE risk and unitary increase in rSS value*



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Correspondence

Interim analysis at 6 months from the LEG-flow Drug Eluting Balloon for the treatment of femoropopliteal occlusions (LEG-DEB) registry

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Patients with lower limb atherosclerosis due to femoropopliteal disease may suffer from critical limb ischaemia or life-limiting claudication and may have multiple comorbidities limiting a surgical therapeutic option. Today, in these cases, balloon angioplasty (PTA) is a valuable therapeutic approach, but its long-term efficacy is hampered by the occurrence of restenosis of the treated arterial segment. This event is even more frequent in patients with critical limb ischaemia (CLI) (Rutherford Class > 4) due to the presence of more pronounced systemic inflammation [1].

The use early generation drug-coated balloons (DCB) has shown promising results in the prevention of post-PTA restenosis in the femoropopliteal artery in randomized clinical trials [2]. However, these trials mostly enrolled patients with claudication (Rutherford Class ≤4) and thus do not provide any data on the device's therapeutic efficacy in CLI patients. Moreover, the devices used in these trials have several unsolved technical limitations such as inconsistent drug coating concentrations, significant drug loss prior to treatment, use of large paclitaxel (PTX) particles which increases the risk of embolization, and excessive initial balloon-artery drug transfer rates resulting in early drug-in-tissue concentrations which are too high. All these limitations reduce the therapeutic efficacy of the drug released onto the arterial wall, and hamper clinical efficacy in challenging conditions (*i.e.* CLI patients).

In order to overcome these limitations, a new generation DCB has been developed, which is covered with a homogenous and stable surface coating using extremely small, non-visible PTX particles, and which does not require the use of an extra DCB protection and insertion tool [3]. There is scant information on the efficacy of new generation DCB in the treatment of femoropopliteal obstructions in "real world" patients (including both patients with claudication and with CLI) [4]. The aim of this multi-centre, prospective registry was to evaluate the safety and efficacy, at six months, of a new generation PTX DCB (LEGFLOW®, Cardionaovum) for the treatment of femoropopliteal artery disease in a "real-world" setting.

From 01/2014 to 06/2015, 123 consecutive patients undergoing PTA of the superficial femoral artery and/or popliteal artery were enrolled in four different European institutions. All patients underwent PTA with LEGFLOW balloons. Among the treated patients, 79 (64.2%) were treated for claudication and 44 (35.8%) for CLI. In total, 76 (61.8%) patients underwent PTA for *de novo* lesions (mean lesion length (MLL) 95.1 ± 57.0 mm), 26 (21.1%) patients for restenosis (MLL 96.1 ± 32.1 mm) and 21 (17.1%) patients for in-stent restenosis (MLL 114.3 ± 24.1 mm).

The primary endpoint of primary patency at 6 months was defined as the absence of clinically-driven target lesion revascularization (TLR) and binary restenosis (>50%) assessed by angiography or duplex ultrasonography (DUS) if angiography was unavailable. Pre-specified secondary endpoints were death, cardiovascular mortality, minor and major amputation, and clinical and haemodynamic success. Patients were evaluated at baseline through angiography, and at 1, 6, 12 and 24 months by a phone call. As required by protocol, an interim analysis was conducted at 6 months, and is reported here.

All procedures were successful in terms of angiographic and clinical success. Technical and procedural success was achieved in all patients. At 6 months, two patients died for non-cardiovascular causes (1.6%). Freedom from TLR was obtained in 88.6% of all patients (Table 1). Freedom from TLR in patients with claudication was obtained in 93.6% and in patients with CLI 79.5%. Analysing the results according to lesion characteristics, freedom from TLR in patients with *de novo* lesions was obtained in 88.1% and in patients with restenosis 80.7%; no TLR was

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Table 1
Efficacy data at six months after baseline procedure.

Freedom from TLVR:	n/N (%)
Overall	109/123 (88.6)
De novo lesions	67/76 (88.2)
Non in-stent restenosis	21/26 (80.8)
In-stent restenosis	21/21 (100)
Critical limb ischaemia	35/44 (79.5)
Claudication	74/79 (93.7)
Diabetics	52/60 (86.7)
Non-diabetics	57/63 (90.5)
Lesions Lenght<100 mm	56/63 (88.9)
Lesions lenght>100 mm	54/60 (90)

observed in patients with in-stent restenosis. Lesion length did not affect TLR rates but the presence of diabetes did.

The data suggests that the use of a new generation, PTX DCB for the treatment of femoropopliteal artery disease represents a safe and effective therapeutic strategy for the endovascular treatment of femoropopliteal obstructions in different clinical (*i.e.* diabetic patients) and anatomic settings (lesion length > 100 mm, restenosis, in-stent restenosis). It should be noted that this study is the first to report the efficacy of a DCB in patients with CLI [4]. In this registry, as expected for this complex patient subset, the LEGFLOW DCB appears to be slightly less successful when compared to the outstanding performance achieved by the DCB in claudicating patients. These data will need to be confirmed with longer-term follow-up.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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Population Trends in Rates of Percutaneous Coronary Revascularization for Acute Coronary Syndromes Associated with the COVID-19 Outbreak

Running Title: *Piccolo et al.; PCI Rates for ACS During the COVID-19 Pandemic*

Raffaele Piccolo, et al.

The full author list is available on page 5.

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Data sharing: The data that support the findings of this study are available from the corresponding author upon reasonable request.

A reduction in hospital admissions for acute coronary syndromes (ACS) has been observed globally in the aftermath of the pneumonia outbreak caused by coronavirus disease 2019 (COVID-19).[1] Despite emergence of anecdotal reports, formal evaluation of variation in percutaneous coronary intervention (PCI) rates during the COVID-19 outbreak has not yet been reported. Italy is one of the countries most heavily affected by the COVID-19 pandemic with 168,941 confirmed cases and 22,170 deaths as of April 5, 2020.

We investigated the association between the outbreak of COVID-19 and PCI rates for ACS in the Campania region, which with 5.8 million residents represents about 10% of the Italian population. Data were obtained from 20 out of 21 PCI centers over an 8-week period, including 4-week before and 4-week after the COVID-19 outbreak corresponding with the first reported case declared by the Civil Protection Department on February 27, 2020. Incidence rates and their ratios were calculated using Poisson regression analysis and interactions for gender and age were estimated by adding the interaction term to the regression models.[2] Population denominators, which were used as offset, were obtained from the Italian census. The ratio change in PCI rates for the entire 8-week interval was estimated by adding a linear term to the Poisson regression. The study was approved by the Ethics Committee of the University of Naples Federico II (Naples, Italy).

From January 30, 2020 to March 26, 2020, a total of 1,831 PCIs were performed in the Campania region; of them 738 (40.31%) were elective PCI (not included), 604 (32.99%) PCI for non-ST-segment elevation acute ACS (NSTE-ACS), and 489 (26.71%) PCI for ST-segment elevation myocardial infarction (STEMI). Mean age was 65.7 years (standard deviation 12), and 804/1,093 PCIs (73.56%) were performed in men. There were no differences in mean age

(65.8±11.8 vs. 65.6±12.2 years, P=0.78) and proportion of men (72% vs. 75%, P=0.29) in the 4-week before the COVID-19 outbreak compared to the subsequent 4-week.

The incidence rate of PCI for ACS decreased from 178 to 120 cases/100,000 residents per-year during the 4-week period before compared with after the COVID-19 outbreak (**Figure 1**). The incidence rate ratio (IRR) was 0.68. The reduction was similar for both NSTE-ACS and STEMI (from 98 to 66 and from 80 to 54 PCI cases/100,000 residents per-year, respectively). The decrease in PCI for ACS was more evident in women (IRR 0.60) than in men (IRR 0.70), resulting in a significant interaction (P<0.001). There was heterogeneity (P-interaction <0.001) in the decline of PCI rates across age categories, with patients <55 years of age less affected by the reduction (IRR 0.75). Findings were consistent between PCI centers in the metropolitan (IRR 0.72) vs. non-metropolitan area (IRR 0.62). Over the interval from week -4 to week +4, the ratio change in PCI rate was 0.51 (95%CI, 0.50-0.52) for ACS, 0.54 (95%CI, 0.53-0.56) for NSTE-ACS, and 0.47 (95%CI 0.45-0.49) for STEMI (**Figure 1**). Compared with the same period in 2019, PCI rates decreased from 190 to 120, from 107 to 66, and from 84 to 54 cases/100,000 residents per-year for ACS (IRR 0.63), NSTE-ACS (IRR 0.62), and STEMI (IRR 0.64), respectively.

In the third most populous region of Italy, we found evidence that the outbreak of COVID-19 was associated with a decline by 32% in the number of PCI for ACS. In the last 2 weeks of the observational period, PCIs for ACS were reduced by 50%. When compared with PCI volumes for the same time in 2019, the decline in PCI rates was of similar magnitude (between 36-38%).

Mechanisms underpinning this decrease are unknown, although several explanations might be involved. Chest pain might be underestimated or misestimated by patients due to the

fear of exposure to COVID-19-affected subjects at hospital admission. This hypothesis might be supported by the stronger decline in PCI rates among women, in whom misdiagnosis and delayed revascularization are more likely to occur in an ACS setting.[3] Other explanations might be related to the unique situation of a country lockdown, potentially leading to less physical activity that might trigger an ACS, coupled with reduced air pollution.

Our data indicate that the COVID-19 outbreak was associated with a remarkable decrease in the rates of PCI across the entire spectrum of ACS. Although we did not measure the hospitalization rates for ACS, PCI represents the most common revascularization modality for ACS patients. The Campania region has been less affected than others by the COVID-19 pandemic and, as a result, no changes occurred during the study period in the regional hub-and-spoke care system and in the management of ACS patients. Therefore, PCI rates effectively reflect ACS rates. However, we cannot determine to what extent the observed trends reflect changes in patient or physician behavior vs. incident ACS.

The findings of this study might have important implications for healthcare systems and suggest that public campaigns aiming to increase awareness of ischemic symptoms should be reinforced during the COVID-19 pandemic. The lack of appropriate and timely revascularization for ACS patients might have other important clinical consequences, not yet measured, including increased risk for heart failure or sudden cardiac death.

Disclosures

None

Sources of Funding

None

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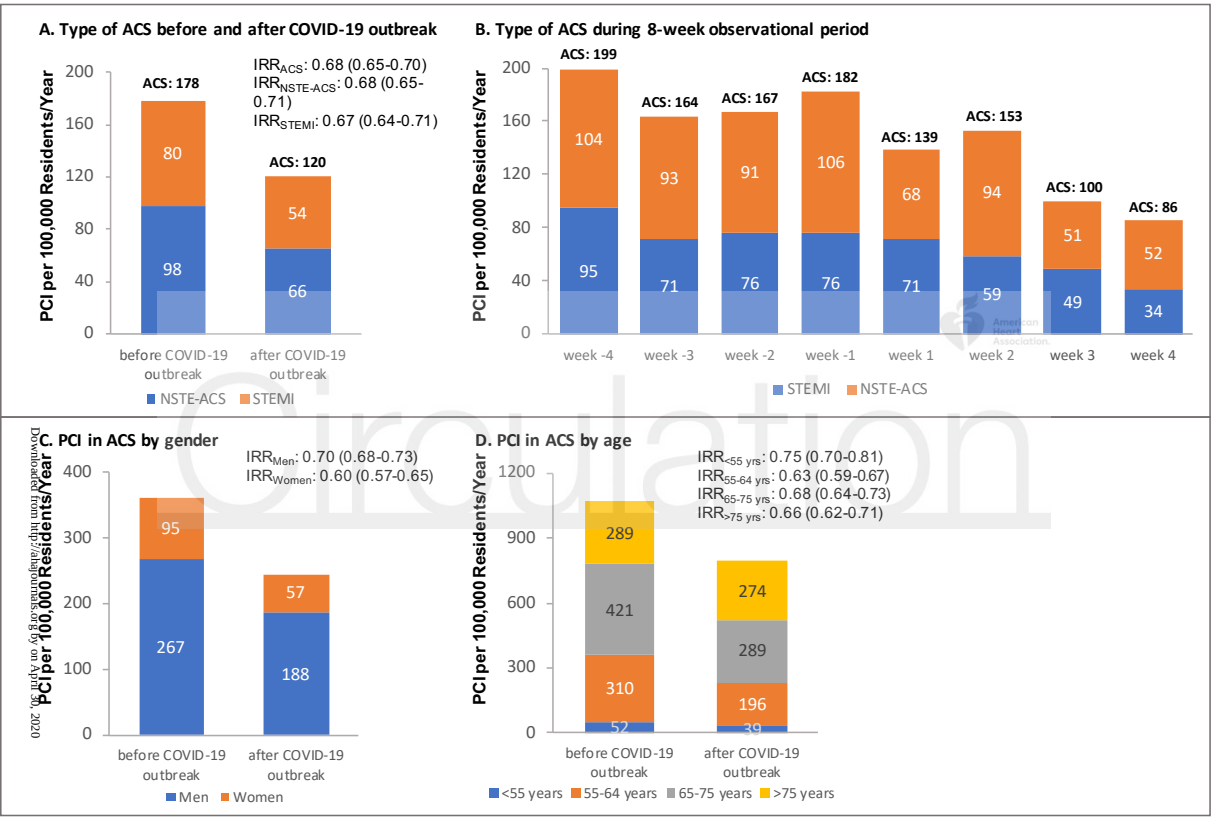
Figure Legend

Figure 1. Panel A. Incidence rates before and after the first reported case of COVID-19 according to the type of ACS. Absolute numbers of PCI cases for ACS, NSTE-ACS, and STEMI were 652, 360, 292 and 441, 244, 197 in the 4-week before and after the outbreak of COVID-19, respectively. In 2019, PCI volumes for the corresponding 4-week period after the outbreak of COVID-19 were 699, 392, 307 for ACS, NSTE-ACS, and STEMI, respectively. **Panel B**. Incident rates by week according to the type of ACS. Weeks -4 to -1 represent the 4-week period before the first case of COVID-19 in the Campania region (Feb 27, 2020) and weeks 1 to 4 represent the 4-week perdiod after COVID-19 outbreak (data were collected until March 26, 2020). **Panel C**. Incidence rates of PCI for ACS before and after COVID-19 outbreak according to gender. **Panel D**. Incidence rates of PCI for ACS before and after COVID-19 outbreak according to age categories. ACS: acute coronary syndrome. IRR: incidence rate ratio. NSTE-ACS: non-ST-segment elevation ACS. PCI: percutaneous coronary intervention. STEMI: ST-segment elevation myocardial infarction.



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Case Report

Management of Transcatheter Aortic Valve Implantation and Complex Aorta Anatomy: The Importance of Pre-Procedural Planning

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Abstract: Aortic stenosis is the most common primary valve lesion requiring surgery or, especially for older patients, transcatheter intervention (TAVI). We showcase a successful transfemoral TAVI procedure in a very high-risk patient and an extremely tortuous S-shaped descending aorta, characterized by heavy calcifications and multiple strong resistance points. We demonstrated that transfemoral TAVI using the "buddy stiff guidewire" technique could be a feasible, simple, quick, and easy procedure able to straighten an extremely abdominal aorta tortuosity. With all techniques available and careful pre-procedural planning, and thanks to the flexibility of new generation TAVI delivery systems, it is possible to safely perform the procedure even in the most challenging patients.

Keywords: transcatheter aortic valve implantation; S-shaped Aorta; aorta tortuosity; buddy wire technique

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1. Introduction

Aortic stenosis (AS) is the most common primary valve lesion in both Europe and North America, requiring surgical or transcatheter intervention, and is rapidly increasing as a result of the aging population [1]. It is a degenerative disease with a prevalence of >7% among patients aged >80 years [2]. Symptomatic severe aortic stenosis has a poor prognosis; in patients who develop symptoms such as exertional dyspnoea, angina, or syncope, the two-year mortality rate is as high as 60% [3]. In fact, there are no medical therapies that have been shown to delay the progression of AS, and aortic valve replacement (either surgical or percutaneous) remains the only treatment that has been shown to improve survival. Therefore, both American and European valve heart guidelines strongly recommend aortic valve replacement (AVR) and early intervention in all patients with a high transaortic echocardiographic gradient (a mean gradient of $\geq$40 mm Hg or a peak velocity of $\geq$4 m/s, class I recommendation), regardless of the left ventricular ejection fraction (LVEF). The best modality of intervention for a patient undergoing AVR for symptomatic aortic stenosis over the years has become increasingly complex, thanks to the continuous technological advances such as minimal access surgery with rapid deployment valves, or the latest generation devices for Transcatheter Aortic Valve Implantation (TAVI). For this reason, the current guideline recommends evaluating all the potential cases of AS in a Heart Valve team as part of a Heart Valve Centre, that carefully consider the decision regarding the indication, timing, and modality of AVR between a surgical aortic valve replacement (SAVR) approach versus TAVI. Since the first human case of a transcatheter aortic valve replacement in 2002 [4], the role of TAVI has expanded from treating inoperable and/or

prohibitive high-risk patients suffering from severe symptomatic AS, through intermediate-risk cases, and now, based on the latest clinical trial results, even low-risk cases. TAVI has clearly become the preferred therapy over SAVR in a setting of high-risk patients with symptomatic and severe AS, a mean Society of Thoracic Surgeon (STS) score of >8% and has been shown to be non-inferior (or even superior when transfemoral access is feasible) to surgical AVR in a clinical setting of intermediate-risk patients with an STS score between 4 and 8% [5]. In addition, results from the latest two randomized trials comparing TAVR with SAVR in low-risk patients (STS score of <4%) have reported that TAVI may also be superior to surgical AVR in the short period of a one-year follow up, although longer-term data on the evaluation of outcomes, especially regarding the valve durability of TAVI, are still lacking [6,7]. In the TAVI procedure, age plays a key role; according to the latest European Society of Cardiology (ESC) guidelines [1], TAVI is mainly recommended for all elderly patients ($\geq$75 years) at higher surgical risk (STS/EuroSCORE II of >8%), or for patients who are not suitable for surgery. In our case, we want to demonstrate that a successful transfemoral TAVI can be performed in a very high-risk patient considered unsuitable for surgery and with multiple severe comorbidities such as active lung cancer and an extremely complex S-shaped anatomy of the descending aorta. We show that TAVI can be performed even in the most difficult patients [8] initially considered contraindicated for aortic valve replacement, thanks to thoughtful planning, careful procedures, and all currently available techniques.

2. Case Presentation

We analyze the case of a 75-year-old male patient who presented to our Cardiovascular Department with chest pain and dyspnoea (New York Heart Association functional class III). He had a recent diagnosis of early stage IIA non-small-cell (NSCLC) lung cancer (considered suitable for chemotherapy and surgery by our oncologist with a 5-year survival rate of 60%). He had a long history of diabetes, hypertension, and also suffered from extreme spinal deformity due to severe scoliosis. The transthoracic echocardiogram showed preserved left ventricular function and severe aortic stenosis with a mean pressure gradient of 48 mm Hg, a valve area of 0.7 cm^2, and a peak aortic jet velocity of 4.3 m/s. In addition to this, the pre-procedural multislice computed tomography (CT) showed extreme tortuosity with an anatomy of the calcified descending aorta with a double S-type curve, adding a more complex scenario to any potential AVR treatment. The extreme tortuosity of the descending aorta is clearly visible in the CT-images both in a coronal view, with an acute angle of 75°, and in the sagittal view, with an acute angle calculated of 95° (Figure 1A–D). As recommended by our oncologist team, an aortic valve replacement is a mandatory procedure in the special case of a patient who has to start chemotherapy treatment for lung cancer. Therefore, he was referred to the Heart Team, where TAVI was considered the most suitable procedure compared to surgical aortic valve replacement (SAVR), in view of the extreme risks of cardiac surgery (calculated STS score was 8.4%, Euroscore II was 24.59%). Through a detailed analysis of multimodal imaging and in particular the CT, it was possible to assess the best way to obtain access (femoral in this case) and to verify the anatomical relationships between the aortic valve and the coronary root or ostia. It was also crucial to choose the optimal device size (we considered in this case a 23 mm SAPIEN 3 Transcatheter Heart Valve-THV for a native annulus diameter of 20 mm and a native annulus area of 400 mm^2) or, as in this case, to plan the optimal technique for crossing the S-shaped descending aorta before the procedure. Given the anatomical tortuosity, we thought that the use of a double stiff guidewire, the so-called "buddy wire" technique, could straighten the descending aorta and reduce any resistance points. On the day of the TAVI, an echo-guided bilateral femoral arterial access (9 Fr on the right side and 7 Fr on the left side) was obtained, together with a 7 Fr left femoral venous access for transvenous pacing. We first performed a standard coronary angiography examination that showed nearly normal coronary arteries. Subsequently, through the left femoral arterial sheath, a 0.035 inch Back-up Meier^{TM} steerable guidewire (Boston Scientific, Marlborough, MA, USA) was inserted

into the proximal descending aorta (Figure 2A) to start stretching the vessel. From the right side, we first placed a Supra Core 35 Hi-Torque 0.035. Extra Support guidewire (Abbott Vascular, 3200 Lakeside Drive, Santa Clara, CA, USA) and, over this guidewire, switched the 9 Fr introducer with a 14 F Edwards eSheath (Edwards LifeSciences, Irvine, CA, USA). Through this sheath, a 0.035 inch INNOWI SX® preformed guidewire (SYMEDRIX GmbH, Oberhaching, Germany) was inserted into the left ventricular chamber. At this point, with the two extra-rigid guidewires within the lumen of the aorta, able to straighten the tortuosity of the vessel, a 23 mm SAPIEN 3 THV was carefully advanced (Figure 2B) and finally deployed under high-speed burst pacing. The Back-up Meier "support" guidewire was held in place until the delivery system was able to pass through the tortuosity points of the abdominal aorta and then quickly removed to avoid some complications, such as aortic dissections or perforations. At the end of the entire procedure, an aortogram demonstrated a good valve position and no evidence of paravalvular leakage or complications (Figure 2C). The patient was immediately taken to the Intensive Care Unit and discharged from the Cardiovascular Department after just three days in NYHA Class I.

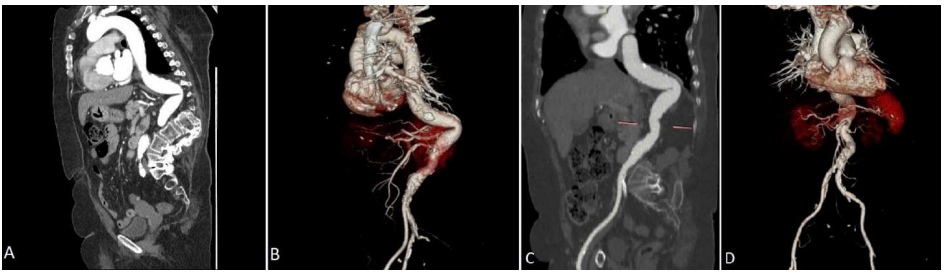

Figure 1. Preprocedural Computed Tomography reconstruction of the arterial tree demonstrating an extreme tortuous descending aorta and spinal cord deformation in a coronal view (**A**) and in a 3D rendering volume view (**B**). Is clearly visible the maximum acute angle of the tortuosity calculated in 75° and the relationship with calcium distribution. (**C**): a sagittal view through the aorta demonstrated a calculated maximum angulation of 96°. (**D**): a 3D rendering volume reconstruction clearly shows the abdominal aorta and surrounded anatomical structures.

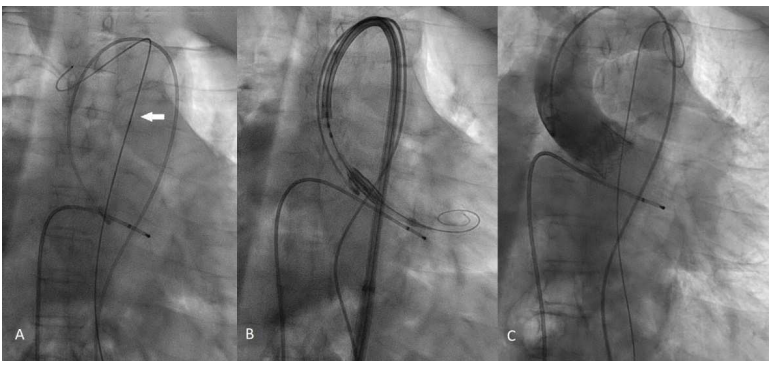

Figure 2. Intraprocedural fluoroscopy steps. (**A**): The Back-up Mayer (Boston Scientific, USA) 0.035 inch stiff guidewire (white arrow) placed along the aortic arch helps to straightens the aorta. (**B**): The Sapien 3 Heart valve prior to deployment; it is clearly visible that the INNOWY SX (SYMEDRIX GmbH) second stiff guidewire is positioned in the left ventricular chamber. (**C**): The aortography clearly demonstrated the correct deployment of the Sapien 3 valve without any evidence of paravalvular leak.

3. Conclusions

There are rare cases [8,9] in the medical literature showing the performance of TAVI with techniques to straighten the vessel in patients with extreme tortuosity of the thoraco-abdominal aorta, such as an "S" conformation, as well as in those at very high surgical risk due to severe comorbidities, such as active lung cancer. As already explained, the decision involving the indication and modality of intervention between SAVR and TAVI procedures deserves careful pre-procedural planning in a very high surgical risk patient such as this. The assessment of operative risk has been facilitated through the use of conventional scoring systems, useful to evaluate the risks of cardiac surgery, such as the Society for Thoracic Surgeons Predicted Risk of Mortality (STS-PROM) and the European System for Cardiac Operative Risk Evaluation (EuroSCORE II) risk calculator systems. However, although these scores are accurate in identifying patients at the highest risk, they were developed to predict the average mortality rate within 30 days after cardiac surgery and have been found to overestimate in-hospital mortality after the TAVI procedure. For this reason, the current guidelines for the management of aortic valve disease recommend considering other risk factors beyond the conventional scores mentioned above in the decision making of patients undergoing TAVI. In this scenario, the assessment of "frailty", defined as a "syndrome of impaired physiological reserve and increased vulnerability to stressors" [10,11], becomes an important predictor of poor outcomes post-TAVI procedure. In the management of complex patients suffering from aortic valve disease, this is very important, as already stated by the recent ESC consensus document, the presence of a multidisciplinary Heart Valve Team as a part of a Heart Valve center hospital. In such context, the indications of the guidelines suggest choosing AVR in patients at low-risk with an STS-PROM or EuroSCORE II score of <4% or EuroSCORE I logistic of <10% and no other risk or technical factors such as frailty, porcelain aorta, or chest radiation sequelae. Patients with associated cardiac conditions requiring concomitant surgery, like severe complex coronary artery disease, ascending aortic aneurysm, severe primary mitral or tricuspid valve disease, septal hypertrophy, or the presence of aortic or left ventricular thrombi, should also be evaluated for a surgical AVR. Some others anatomical features, like the presence of an unsuitable aortic root anatomy with low coronary heights (defined as a distance from the coronary ostium to the aortic valve anulus of <12 mm), or an extreme annular dilatation, or a dysmorphic valve morphology with a presence of a bicuspid aortic valve leaflets with various degree and pattern of calcification, should all be considered from the Heart team suitable for SAVR. On the other hand, according to the latest guideline, TAVI is recommended for patients judged unsuitable for SAVR by the Heart Valve Team (Class I of the ESC guideline), particularly patients at higher surgical risk (STS-PROM or EuroSCORE II score of $\geq$8% or EuroSCORE I logistic of $\geq$10%), and especially elderly patients (>75 years) with suitable access for transfemoral TAVI. Despite the possibility of alternative peripheral accesses (transapical, trans-subclavian, etc.,) we considered, like all the current guidelines, the femoral access as the gold standard for TAVI and we recommend reconsidering surgery when this access is not feasible [12]. For this purpose, we chose a balloon-expandable Sapien3 THV prosthesis, that is associated with lower rates of pacemaker implantation after the procedure, and its Ultra-Low profile 14 Fr Edwards eSheath (Edwards LifeSciences, Irvine, CA, USA) introducer system. This sheath expands transiently during the passage of the Sapien 3 THV facilitating the placement of the large femoral sheath into the atheroma-altered arterial wall of the iliaco–femoral axis and the extremely tortuous descending aorta of the patient. The assessment of the Heart Team, consisting of a cardiac surgeon, an interventional cardiologist, a clinical cardiologist, and cardiac imaging is therefore crucial in this complex scenario not only to choose the best treatment option for the patient but also for careful pre-procedural planning. In this case, TAVI was the preferred procedure over SAVR because the combination of STS/EUROSCORE of >8 points, severe aortic wall calcifications, an age of >75 years, and the frailty of the patient with coexisting active lung cancer were all features in favor of transcatheter aortic valve implantation [13,14]. Vessel anatomy that was thought to be prohibitive even just a few years ago can be overcome

thanks to improvements in delivery system technology, better procedural planning, and a greater operator experience. Of course, caution must always be taken in these extreme cases because tortuosity can predispose to rupture or arterial wall dissection, which can be a dramatic event when the aorta is involved. In particular, the presence of calcifications (some calcified atherosclerotic plaques are clearly visible in Figure 1B,D in the 3D-rendering CT view along the descending aorta and both the iliac and femoral arteries) may increase the risk of rupture or dissection due to the reduced compliance of the aortic wall. Fortunately, in the literature, the reported incidence of acute aortic dissection during TAVR is a rare complication involving about 0.2% of cases [15]. We routinely perform an aortography of the ascending aorta at the end of the procedure to avoid the above complications. In a case like this, it is very important to choose the best treatment option for the patient to give them a chance of surviving lung cancer. The extremely tortuous descending aorta was characterized by several points of strong resistance, which led to great difficulty in pushing and delivering the valve device. For this reason, and thanks to an accurate analysis of the pre-procedural angio–CT images, we have previously considered in the Heart Team performing TAVI with the help of the so called "Buddy Wire" technique, an approach derived from the experience of treating severe calcifications in percutaneous coronary interventions (PCI). The Buddy Wire technique consists of a second guidewire (usually a 0.014 inch guide wire) placed next to the one used to advance balloons and stents into the coronary artery during the percutaneous coronary intervention (PCI). The "Buddy Wire" is a simple, fast, and readily available procedure. In this case, the use of a second 0.035 inch "stiff" guidewire (instead of a 0.014 inch coronary guidewire), alongside the first wire used to advance the valve device during TAVI, should be chosen to straighten an extremely abdominal aorta tortuosity. Obviously, this technique is useful only in the case of severe complex aorta anatomy, while the presence of a second stiff guide wire lying in the aorta without necessity can lead to some complications such as aortic dissection or perforation. Transfemoral TAVI with the "Buddy double-stiff guidewire" (as we have named it) technique can be feasible only if correctly used [16–19]. With this approach, we demonstrate that TAVI can be performed substantially safer and can reduce the likelihood of procedural complications even in high-risk patients with a very complex anatomy. With all existing techniques and thanks to the flexibility of the new generation TAVI delivery systems, it is possible to safely perform this procedure even in the most difficult patients, who were initially considered contraindicated to aortic valve replacement.

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Institutional Review Board Statement: In this work we presented a case report of a single patient who underwent percutaneous TAVI in order to solve a specific clinical life-threatening condition. No research or experimental protocols were applied to animal or human subjects that mayrequire scientific ethical approval.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data regarding this clinical case can be provided by the authors following reasonable request.

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Discussion and concluding remarks

Part I: In vitro strategies to improve endothelial function and response to ischemia

The role of endothelial cell dysfunction in the development of atherosclerotic disease and in the response to ischemia has proven to be critical. Finding some target for drug development targeting endothelial dysfunction is crucial for prevention and treatment of ischemic cardiomyopathy and peripheral hindlimb ischemia. I have focused my researches on the role of mitochondrial dysfunction and on the role of circulating Ig, FcIgR and C Reactive Protein.

Oxidative stress mediated by reactive oxygen species (ROS) plays a striking role in cardiomyocyte survival and in the pathogenesis of heart left ventricular dysfunction, particularly after myocardial infarction (36-38). ROS directly alter vascular function and cause changes in vascular tone by several mechanisms including altered NO bioavailability or signaling (39,40). ROS- producing enzymes involved in the increased vascular oxidative stress observed during hypertension include

the NADPH oxidase, xanthine oxidase, the mitochondrial respiratory chain and an uncoupled endothelial NO synthase (41,42). Oxidative stress seems also to have a pathophysiological role for arterial hypertension and vascular remodeling (41). Mitochondria are a critical intracellular source of ROS, and while oxidative phosphorylation plays a limited role in endothelial cells metabolism, mitochondria are crucial for endothelial cells signaling and function (42). Mitochondria also plays a critical role in postischemic neovascularization promoting VEGF-mediated activation of Akt signaling (43).

We demonstrate in our experiments the critical role of Akap1 protein, a mitochondrial A kinase anchor protein, in the modulation of endothelial cell function, by interacting with Akt kinase, affecting mitochondrial survival, ROS production, NO-dependent vascular relaxation, reduced postischemic neovascularization, and increased blood pressure. This is the first evidence that alterations in mitochondrial cAMP-dependent signalling might affects endothelial cells behaviour and vascular reactivity in vitro and in vivo, while the role of cAMP-mediated signalling have been previously involved in several

cardiovascular processes and diseases. Akap1 also participates in VEGF signalling through PKA-dependent phosphorylation of VEGF receptor II. VEGF-induced Akt phosphorylation was significantly reduced in Akap1−/− endothelial cells compared with wild type cells, suggesting that downstream effectors of VEGF pathway are altered in Akap1−/− endothelial cells. We observed reduced Akt activation in Akap1−/− endothelial cells also in response to hypoxia. Finally, Akap1 might regulate eNOS activity through its effects on Akt. Hence, Akap1 deletion might result in reduced Akt phosphorylation and, in turn, eNOS activation. All these observations prompt the focus on the potential use of mitochondrial signalling as therapeutic target for treatment of cardiovascular disease. While scavenger based approached to reduce injury from chronic oxidative stress have given alternate results (6), specific strategies addressing mitochondrial ROS and activators of AMP-activated protein kinase/peroxisome proliferator-activated receptor γ coactivator 1-α have shown promising results also in endothelium (44,45). Pharmacological approaches that enhance Akap1 mitochondrial

targeting may provide beneficial effects in CVD through a broad spectrum of cell-specific effects.

We demonstrated that that chronic reduction of circulating IgG, observed in patients with common variable immunodeficiency, is associated with impairment of endothelial mediated vascular reactivity and that such impairment can be corrected in the short term by intravenous infusion polyclonal IgG (which is part of the chronic therapy for these patients). In vitro cell experiments demonstrated that IgG induce NO production in endothelial cells. Circulating IgG also were capable of affecting insulin action, as indicated by the powerful effect of polyclonal IgG infusion on circulating serum insulin concentration and whole-body insulin sensitivity. Notably, there is no demonstration that patients with common variable immunodeficiency present an increase rate in cardiovascular disease mortality and morbidity, however it should be noted that they generally die for infectious or neoplastic complications in youth when not adequately treated with substitutive infusions. The observed improvement in insulin sensitivity induced by IgG infusion does not find a mechanistic explanation in our studies, not being clearly linked

to improved vasodilation, and can probably be explained by the results of a recent mice study which demonstrated that circulating IgG favours an improvement in the endothelial-mediated endocytosis process that favours the transit of insulin from the vessel lumen to the interstitial space and makes more insulin available for the peripheral tissues (19). Finally we observed that C-reactive protein progressively, although not significantly, declines after the infusion of IgG. It is conceivable that such reduction somehow might affect in vivo endothelial function or insulin sensitivity, taking into account that this protein interact with FcRs on the endothelial cells. However we observed a direct effect of IgG on endothelial cells in vitro and an hyperacute effect, unlikely to be referable to C-reactive protein, on endothelial-dependent vasodilation in vivo. Obviously, this is a preliminary study that does not provide a pathophysiological explanation for the previously described observations. However this axis could be promising as therapeutic target for new cardiovascular drug development.

Part II: Clinical strategies to improve the outcome after percutaneous interventions

Since its beginning, interventional cardiology has showed fantastic technological improvements over the past twenty years. The progressive reduction in required sheat caliber, the widespread diffusion of radial access, continuous advances in pharmacological periprocedural management and the improvement in quality of radiographic acquisitions are only the first accountable examples. The continuous refinements in stent type and design with the transition from early to new-generation devices led to a dramatic decrease of the rate of failure, over time, after percutaneous revascularization, conversely with a dramatic improvement in safety profile, deriving from thinner struts and modification in polymer coating, which allows, in most cases, a reduction in the strength of antiplatelet therapy switching from dual antiplatelet therapy (DAPT) to a single drug, after 1-3 months from procedure (25,26). These ameliorations are also determining a shift, in the context of tailored antiplatelet regimen, to the idea that prolonging DAPT duration improves ischemic prognosis, almost in most patients,

especially those without high bleeding risk, as observed in the DAPT and the PEGASUS-TIMI 54 trial (46,47), to a paradigm of shortening DAPT duration to avoid bleeding events which prognostic impact has recently been judged comparable to that related to ischemic complications (32). In this context, the results of TWILIGHT (48) and the MASTER-DAPT (49), two trials evaluating safety of a short-DAPT regimen in patients at high bleeding risk or high ischemic risk in the TWILIGHT and patients at high bleeding risk in the MASTER-DAPT, deserve a mention. Patients were subjected to 3 months of DAPT followed by ticagrelor monotherapy till to one year in the TWILIGHT trial and one month of DAPT followed by two months of monotherapy in the MASTER-DAPT, being compared respectively to one year and three months DAPT regimen in the control group (48,49). In the TWILIGHT, among high-risk patients who underwent PCI and tolerated 3 months of DAPT, ticagrelor monotherapy was associated with a lower incidence of clinically relevant bleeding than ticagrelor plus aspirin, with no higher risk of death, myocardial infarction, or stroke (48). In the recent MASTER DAPT trial, which results were showed in the

last European Society of Cardiology annual congress, one month of DAPT revealed to be noninferior to the continuation of therapy for at least two additional months with regard to the occurrence of net adverse clinical events and major adverse cardiac or cerebral events and a lower incidence of major or clinically relevant nonmajor bleeding (49). These results were at least in part counteracted by those from the results of another randomized clinical trial, the STOP DAPT 2 ACS, in which patients with acute coronary syndrome were randomized to classic 12 months DAPT or to one month of DAPT followed by clopidogrel monotherapy till to one year from the event (50). In this study, 1-month of DAPT with subsequent 11 months of clopidogrel monotherapy did not result non-inferior to 12 months clopidogrel-based DAPT for the net combined primary endpoint of CV death, myocardial infarction, stent thrombosis, stroke and TIMI major/minor bleeding (50). Short DAPT was associated with a lower incidence of severe bleeding (type 3 and 5 according to BARC classification, 0.54 % vs. 1.31 %), at the cost of increasing the risk of myocardial infarction (1.59 % vs. 0.85 %) (50). This apparent incongruence may be explained by

different populations enrolled (high bleeding risk versus post acute coronary syndrome setting). Together with improvement in technique, one should never forget that the treatment has to be focused on the disease and not to the stent. Refining in the outcome of percutaneous revascularization should also rely on the optimization of the final results by intravascular imaging, which role will probably become critical in the next future, and also on adequate lesion preparation obtainable with actually available debulking devices (especially for calcified lesions) and on adequate use of mechanical circulation support devices were indicated by the clinical context. Sometimes the progress meets some stubbing stones during the route, as happened with bioresorbable stents, which were considered a Copernican revolution in the first year of 2010 century but, after brief observations, appeared to carry much more troubles and concerns than advantages. No one can exclude, however, their return after a necessary improvement of the technology in a near future.

As previously discussed, significant improvement have also been reached in the treatment of acute myocardial infarction,

particularly in the setting of its most worrisome expression, STEMI. As discussed previously, a residual field of uncertainness is the correct management of residual coronary stenosis after execution of primary percutaneous coronary intervention. While a significant number of trials (such as the PRAMI, the CVLPRIT, the DANAMI-3-PRIMULTI and the COMPARE ACUTE (51-55)) have clearly shown that complete revascularization should become a dogma for these patients, at higher risk of future cardiovascular events, the correct management remains controversial. Being the prognostic improvement mostly related to prevention of successive myocardial infarction and clinically driven revascularizations, a correct definition of the lesions to be treated appears mandatory. Issues remain regarding the correct timing and guiding criteria for interventions. To avoiding a rely on subjective visual estimation, functional evaluation for the presence of inducible ischemia by invasive pressure wire based devices with the classic FFR or with newly validated resting index such as iFR has been proposed and seems to be feasible and effective, even with some concern when performed in the hyperacute phase

(56,57). In last years wave-free functional evaluation tools, such as quantitative flow ratio, are gaining a rising attention with some initial validation study (58). Results of a recent trial (the FLOWER-MI) are concerning. In this study 586 patients with STEMI and multivessel disease underwent FFR-guided revascularization and 577 other patients in the same clinical context underwent angiography-guided revascularization (59). During follow-up, a primary outcome event (a composite of death from any cause, nonfatal myocardial infarction, or unplanned hospitalization leading to urgent revascularization at 1 year) occurred in 32 patients (5.5%) in the FFR-guided group and in 24 of 577 patients (4.2%) in the angiography-guided group (hazard ratio, 1.32; 95% confidence interval, 0.78 to 2.23; P=0.31) (59). FFR-guided strategy did not show a significant benefit over the angiography-guided strategy with respect to the risk of death, myocardial infarction, or urgent revascularization at 1 year (59). While, given the wide confidence intervals for the estimate of effect, the findings do not allow for a conclusive interpretation, it is possible that other factors than the ischemic burden of the lesion have to been taken into account for an

adequate and correct decision making. One of them could be the "prognostic" relevance of the lesion. In our analysis we validated the Added Index, previously designed to foresee the results of an eventual FFR analysis of a coronary stenosis in a stable setting, as an independent prognostic factor after STEMI. Residual Added Index after primary revascularization >2.23 identifies those patients presenting with STEMI and multivessel disease who would benefit the most of complete myocardial revascularization to avoid the demonstrated increased risk of major adverse cardiac events, defined as the composite of all-cause death, myocardial infarction, clinically driven revascularizations, and non-culprit vessel oriented clinical events, defined as the composite of all-cause death, non-culprit vessel related myocardial infarction and clinically driven revascularizations. Use of an easy to calculate angiographic index favours the avoidance of invasive manipulation with pressure wire, which could be desirable in some setting, reducing the risk of performing clinically unnecessary coronary interventions. The relevant potential role for prognostic stratification has also been confirmed in our preliminary

analysis of comparison between residual Added Index and residual Syntax Score –i.e., the Syntax Score deriving from residual stenosis after primary PCI and left untreated, which is a known prognostic predictor (60)- in patients undergoing culprit vessel revascularization after STEMI which demonstrates a good correlation among high residual Syntax Score and high residual Added Index, which is, to be noted, a bit faster to be calculated.

As previously underlined, bleeding events represent a detrimental factor in prognosis of patients with acute coronary syndrome and/or subjected to percutaneous revascularization (32). We observed that in-hospital hemoglobin drop of >3 g/dl, even in the absence of overt bleeding, showed a continuous, direct association with increased 1-year mortality. About 6% of patients enrolled in the MATRIX trial had a significant hemoglobin drop without a clinically relevant hemorrage. Multiple mechanisms can be responsible for in-hospital hemoglobin drop, such as antithrombotic treatments, invasive procedures, arterial access, occult bleeding (from gastrointestinal tract), intense inflammatory status, hemodilution

or bone marrow impairment. Anemia may impair prognosis by worsening myocardial ischemic insult, decreasing the oxygen supply to the jeopardized myocardium, increasing myocardial oxygen demand due to higher cardiac output status and inducing abnormal neurohormonal activation and cardiac remodeling. This MATRIX trial subanalysis is the first study addressing the prognostic impact of hemoglobin reduction per se, without overt bleeding in the setting of acute coronary syndrome. Regardless of baseline hemoglobin, a hemoglobin drop >3 g/dl, even in the absence of detectable bleeding, appears prognostically relevant and may be represent a valid surrogate endpoint for future studies and trials, also taking into account that bleeding events considered prognostically relevant (such as BARC type 3 to 5) are relatively infrequent limiting study power. The identification of predictive factors for a significant hemoglobin drop such as advanced age, female sex, reduced body mass index, peripheral vascular disease, diabetes mellitus, particularly when insulin-dependent, advanced Killip class, and tachycardia, and the observation that the experimental strategies analyzed in the MATRIX trial (radial access and bivalirudin infusion (61,62))

were able not only to reduce overt bleeding events but also significant hemoglobin drop led to conclude that identification of high bleeding risk patients since their admission and their adequate treatment to avoid hemoglobin loss are critical to improve prognosis.

Drug coating devices has significantly improved long-term outcomes in a complex setting such as the angioplasty in the femoropopliteal arteries (35). The results of our real-world registry deserve a special attention, because lesions and procedures of clinical trials do not necessarily represent the standard of care or diverse lesion morphologies in common clinical practice. The present study enrolled a patient population more typical of routine clinical practice, including high percentages of patients with advanced disease (Rutherford category 4/5), intermediate length lesions, and restenotic lesions. The good efficacy results observed at six and twelve months follow up were counterbalanced by no safety issue. An important clinical observation from this study was the high percentages of in-hospital major cardiovascular events in

patients with critical limb ischemia, which highlights the need to improve clinical and interventional care in this frail population.

A final message deriving from all these studies could be summarized in the concept that atherosclerosis needs to be struggled with a multilevel approach, starting with a deep comprehension of its basic mechanisms (most of them, such as the role of endothelial dysfunction, still requiring an adequate study) and proceeding to a constant refining in weapons and technologies used in interventional procedures with the aim to improve efficacy and safety but also taking into account the appropriateness of invasive procedures to avoid futility and iatrogenic injury.

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Second operator in the cath-lab for coronary angiography, PCI in stable patients or patients with acute coronary syndrome, primary PCI. Use of intravascular imaging and FFR. Night and weekend availability for urgent coronary angiograms/PCI or transvenous temporary pacing. Percutaneous aortic valve replacement (TAVR) with use of the following devices: CoreValve Evolute R, SAPIEN 3 and SYMETIS ACURATE NEO. Right heart catheterization. Percutaneous closure of patent foramen ovale, atrial septal defect, left atrial appendage. Angiography and PTA for lower limb arterial disease and carotid artery disease. Percutaneous treatment of addominal aorta aneurysm. Attending about 1.000 invasive procedures.

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MOTHERTONGUE **ITALIAN**

OTHER LANGUAGES

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• SPOKEN EXPRESSION	GOOD

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- Excellent use of Microsoft Windows, Microsoft Word, Micorsoft Excel, Microsoft Power Point and Apple System.
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- Excellent use of Osirix
- Italian guide license.
- Italian nautic license.

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LIST OF PUBBLICATIONS (FROM PUBMED)

1: Intorcia A, Ambrosini V, Capasso M, Granata R, Magliulo F, Carbone GL, Capobianco S, Rotondi F, Lanni F, Manganelli F, Di Lorenzo E. Management of Transcatheter Aortic Valve Implantation and Complex Aorta Anatomy: The Importance of Pre-Procedural Planning. Int J Environ Res Public Health. 2022 Apr 14;19(8):4763. doi: 10.3390/ ijerph19084763. PMID: 35457629; PMCID: PMC9025825.

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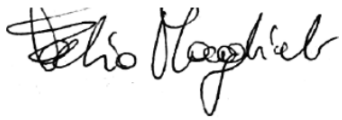

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