Second drug-eluting stent implantation versus coronary artery bypass grafting in treatment of young patients with left main and/or multivessel coronary disease

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Abstract

Background Many studies have compared outcomes of coronary artery bypass graft (CABG) and percutaneous coronary intervention (PCI) for complex coronary artery disease (CAD). However, no trials have focused on young patients (< 45 years) with complex CAD. We conducted a retrospective evaluation to compare the outcomes of a 2nd drug-eluting stent and CABG in young patients with LM or three-vessel disease. Methods In the young patients with complex CAD who underwent PCI or CABG, Kaplan-Meier analysis and Cox regression before and after propensity-score matching were used to compare major adverse cardiac and cerebrovascular events (MACCE), including myocardial infarction (MI), stroke, death and repeat revascularization. Results During the follow-up, MACCE occurred in 20.5% of patients in the PCI group and 8.6% in the CABG group (hazard ratio HR: 3.263, 95% confidence interval CI: 1.379 to 7.722, p=0.007). Repeat revascularization occurred more frequently in the PCI group (18.9% vs. 3.7% respectively, HR: 6.968, 95% CI: 2.036 to 23.842, p=0.002). There were no significant differences in other endpoints. After propensity-score matching, no conclusions were changed. Conclusions In young patients with LM or three-vessel disease, PCI showed a higher incidence of MACCE, which was mainly driven by repeat revascularization. However that did not translate into hard endpoints differences. Therefore, PCI is an alternative treatment to CABG in young patients with complex CAD.

Background

The left main (LM) disease and three-vessel disease are complex coronary artery disease (CAD), whose treatment is more difficult. Coronary artery bypass graft (CABG), as an effective treatment for CAD, has introduced more than 50 years which, is currently the preferred modality to treat complex CAD (1-3). However, over the last twenty years, there
have been significant advances in percutaneous coronary intervention (PCI), from the era of balloon angioplasty and subsequently bare-metal stents to drug-eluting stents (DESs) (4-6). With improving technology and technique of PCI, such as adjunctive antithrombotic drugs, periprocedural management and the experience of interventional cardiologists, research has increasingly focused on more complex diseases, such as disease and multivessel disease.

The latest European Society of Cardiology (ESC) and European Association for Cardio-Thoracic Surgery (EACTS) guidelines(7) recommend CABG (class I, level A) for complex CAD both LM disease and three-vessel disease regardless of anatomic complexities of coronary arteries. However, PCI is an alternative in the case of LM disease and three-vessel disease if the SYNTAX score is ≤22 (class I, level A); if the SYNTAX is >22, it would be inferior for LM disease and three-vessel disease (class II or III, level A or B). Even so, along with the rapidly progressing technology, an increasing number of patients and cardiologists prefer PCI over CABG, according to many trials. Several trials have reported that PCI is noninferior to CABG in patients with LM disease (8) or multivessel disease (9). On the other hand, many trials have suggested CABG might provide better clinical outcomes than PCI (10-12).

In view of the younger characteristics of CAD patients and the likelihood of graft failure, and considering that 62% of patients will have recurrent ischemia by 15 years postoperatively (13), it is essential to consider the risk/benefit ratio of PCI and CABG for LM and three-vessel disease, weighing procedural invasiveness and the associated short-term complications against long-term event rates of death, myocardial infarction (MI), stroke, and repeat revascularization, and improvements in health-related quality of life (14). Therefore, we hypothesize that PCI should be performed at an early age if possible to alleviate symptoms to provide another choice for advanced illness. We conducted a
retrospective evaluation to compare real world outcomes between CABG and PCI using second-generation DESs in young patients with LM disease or three-vessel disease.

Methods

This was a single-center retrospective study comparing PCI with 2nd DES and CABG in young patients with LM and three-vessel disease. We performed a review of all young patients who underwent diagnostic coronary angiography, and PCI or CABG at Beijing Anzhen Hospital from January 2015 to December 2016. We screened these patients and enrolled them if they had LM and/or three-vessel disease and underwent PCI with a 2nd-generation DES or CABG. The patients who had previously undergone PCI were also included in our study. Patients were excluded if: 1) they did not suffer from LM or three-vessel disease; 2) they had acute myocardial infarction (MI), either ST segment elevation or non-ST segment elevation; 3) they underwent concomitant valvular or aortic surgery; 4) they had previous CABG surgery; and 5) patients were unable to receive both procedures or did not want to.

In our trial, the revascularization strategy was determined by physicians’ and/or patients’ preferences, on the basis of hemodynamic conditions, anatomic factors, vessel size, the presence of comorbidities and quality of arterial and/or venous conduits grafts fit.

All PCI procedures were performed according to current standard interventional guidelines. Antiplatelet therapy and periprocedural anticoagulation followed standard regimens. All patients received 300 mg of loading dose aspirin and/or clopidogrel (or 180 ticagrelor) before the procedure. After PCI, all patients were recommended 100 mg/day aspirin indefinitely and 75 mg/day clopidogrel or ticagrelor 90 mg twice daily for at least 1 year. There was no restriction on the second-generation DESs.

The bypass graft revascularization was performed with standard bypass techniques. A normal midline sternotomy incision was used to expose the heart and both on-pump and
off-pump surgeries were performed at the preference of the surgeon. The internal thoracic artery was preferred for bypass of the left anterior descending artery. After CABG, medications were given according to the guidelines or the preference of the surgeon. Patient data on demographics, comorbid conditions, laboratory echocardiography, procedures and so on were collected via chart review. The ethics committee at our hospital (Beijing Anzhen Hospital) reviewed our study protocol and approved the use of clinical data for the study. Because of the retrospective nature of our trial, the need for patient informed consent was waived. Follow-up and information on the clinical status were achieved by clinical visits and telephone interviews.

The primary endpoint in our trial was major adverse cardiac or cerebrovascular events (MACCE), a composite of all-cause death, stroke, myocardial infarction (MI), and repeated revascularization. Secondary endpoints were the individual occurrence of all-cause death, stoke, MI and repeat revascularization. Deaths were considered cardiac unless unequivocally noncardiac. Stoke was defined as a focal neurological deficit of central origin lasting >24 h, confirmed by a neurologist and computed tomography or magnetic resonance imaging. MI was defined as a creatine-kinase-MB level >50 ng/ml or the appearance of new Q-waves or ST segment elevation >2 mm, on the electrocardiogram which were confirmed by a veteran cardiologist. Repeated revascularization in our trial was any revascularization that was performed on any vessel by PCI or CABG.

Young patients were defined as < 45 years old according the World Health Organization (WHO). The lesion of each vessel, including its main branches (diameter ≥1.5mm) was defined as ≥50% stenosis. Multivessel disease or three-vessel disease was ≥50% stenosis in all three epicardial coronary arteries, consisting of the left anterior descending artery (LAD), left circumflex artery (LCX) and right coronary artery (RCA) or their main branches. Other definitions, such as hypertension (HT) and diabetes mellitus, were based on the
basis of international guidelines or medication management.

Baseline and outcome data for individual patients were pooled. Continuous variables are expressed as mean±standard deviation (SD) and were compared using Student’s T-test. Categorical variables are presented as frequencies and proportions and comparisons were performed using the chi-square test or Fisher’s exact test, as appropriate.

The rate of cumulative events and incidence curves for clinical outcome were estimated using the Kaplan-Meier method. A Cox proportional hazard regression was performed to determine independent predictors of MACCE, MI, stroke, death and repeat revascularization in univariate and multivariate analyses. The analyses were used to determine the noninferiority of PCI and to find the adjusted hazards. Adjusted covariates included operation strategy (PCI/CABG), age, sex (male/female), smoking (yes/no), alcohol (yes/no), body mass index (BMI), HT (yes/no), DM (yes/no), hypercholesterolemia (HC, yes/no), family history (yes/no), creatinine clearance rate (CCR), uric acid (UA), C-reactive protein (CRP), blood group (A,B,AB, O), left ventricular ejection fraction (LVEF), prior MI (yes/no), prior heart failure (HF, yes/no), prior stroke (yes/no), prior stent (yes/no), collateral circulation (yes/no), lesion type (LM or three-vessel disease) and Gensini score. Multivariable predictors of outcomes were identified using forward stepwise selection with a significance level of <0.05 for entry and exit criteria. Finally, the significant factors and several clinically important factors entered subsequent analysis. The results are reported as hazard ratio (HR) and 95% confidence interval (CI).

To reduce selection bias and any other related potential confounding factors, we performed a baseline characteristic adjustment for patients using propensity score. The propensity scores were estimated using a multiple logistic regression model. A full nonparsimonious model was developed that included all variables listed in Tables 1 and 2. Patients who underwent PCI and patients who underwent CABG were then matched at a
1:1 ratio by propensity score using a nearest-neighbor matching algorithm with a caliper of 0.01. The absolute standardized differences of variables included in the calculation of propensity score were compared before and after propensity-score matching. Standardized differences <10% for these included variables indicated a relatively better balance. The baseline characteristics and outcomes between the two propensity-score-matched subsets were recompared.

Subgroup analyses, including sex, HT, DM, prior MI, prior HF, lesion type and Gensini score, were run using the Cox proportional hazard model. The variables included in the model were significant and were proved in the model design or were clinically relevant. Texts for interaction were performed to assess the heterogeneity of the treatment effect among subgroups. In addition, we utilize the Cox proportional hazard regression to explore the effects of intravascular ultrasound (IVUS) / optical coherence tomography (OCT) and kissing balloon on MACCE that suffered from LM disease and subsequent PCI.

A two-side p value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were carried out using SPSS software version 20.0 (IBM Corporation) or Stata version 14.0 (StataCorp, College Station, TX, USA).

Results

Between January 2015 and December 2016, a total of 1964 patients underwent angiography and PCI or CABG in our hospital. However, 1751 patients were removed from our trial by the inclusion and exclusion criteria, leaving 213 patients who entered the study, including 81 CABG and 132 PCI with 2nd DESs (Figure 1).

Baseline demographic characteristics are listed in Table 1. In the overall population, the incidence of smoking (72.7% vs. 56.8%, p=0.017), hyperlipidemia (22% vs. 8.6%, p=0.012), and several physical and biochemical indexes such as BMI (28.05±3.75 vs. 26.80±3.18, p=0.013), CCR (130.00±32.08 vs. 121.34±27.41, p=0.045), and UA
(390.03±90.05 vs. 347.56±97.86, p=0.001) were higher in patients in the PCI group, whereas the Gensini score (69.80±35.10 vs. 86.64±36.77, P=0.001) showed the opposite difference. The antiplatelet drug $P_2Y_{12}$ inhibitor (99.2% vs. 91.4%, p=0.005) was significantly more common in patients in the PCI group compared with those in the CABG group. There were no differences between treatment groups in terms of age, sex, HT, DM, LVEF, prior MI, prior HF, lesion type or other factors.

The median duration of follow-up among all patients was 38 months (interquartile range: 36 to 41 months). The cumulative incidences of clinical outcomes of all patients are described in Table 2 (see Additional file 1) and Figure 2. Between the follow-ups, the incidence of MACCE was 20.5% in the PCI group and 8.6% in the CABG group (unadjusted HR: 2.508, 95% CI: 1.091 to 5.762, p=0.03; adjusted HR: 3.263, 95% CI: 1.379 to 7.722, p=0.007). The incidence of repeat revascularization was 18.9% in the PCI group and 3.7% in the CABG group (unadjusted HR: 5.435, 95% CI: 1.64 to 18.011, p=0.006; adjusted HR: 6.968, 95% CI: 2.036 to 23.842, p=0.002). There were no significant differences in other endpoints, such as MI, stroke or death, before or after adjusting for multiple variables and clinical background (1.5% vs. 2.5%, p=0.86; 0 vs. 2.5%, p=0.881; 0 vs. 2.5%, p=0.939, after adjustment, respectively).

After propensity-score matching was performed, there were 46 matched pairs of patients in the two groups. The baseline characteristics of patients after propensity-score matching are described in Table 3, and no significant differences were found between the two groups with more variables showing standardized differences less than 10% that an additional figure file shows this in more detail (see Additional file 2). Compared with the CABG group, the PCI group had a higher prevalence of MACCE (32.6% vs. 10.9%, HR: 4.496, 95% CI: 1.592 to 12.695, p=0.005) and repeat revascularization (30.4% vs. 4.3%, HR: 11.6, 95% CI: 2.449 to 55.51, p=0.002) after adjustment. However, as in the overall
population, there were no significant differences in the incidence of MI (2.2% vs. 2.2%, p=0.55), stoke (0 vs. 4.3%, p=0.658) or death (0 vs. 2.2%, p=0.876) (Table 4, Figure 3). Subgroup analyses were performed based on important baseline characteristics. There were no significant interactions between any treatment effects of PCI versus CABG in the rate of MACCE except for the prior MI (p for interaction=0.031). PCI was associated with an increased risk of MACCE in other subgroups. However, the patients who had ever suffered MI experienced a lower rate of MACCE in the PCI group than in the CABG group (HR: 0.652, 95% CI: 0.125 to 3.397) (Figure 4). In twenty-six LM disease patients who suffered from PCI, there were only six conducted IVUS / OCT and seven performed kissing balloon with no significant difference in MACCE (16.7% vs. 25%, HR: 1.607, 95% CI: 0.188 to 13.672, p=0.665; 14.3% vs. 26.3%, HR: 0.498, 95% CI: 0.058 to 4.267, p=0.525, respectively).

In the multivariate Cox regression analysis, age (HR: 1.147, 95% CI: 1.004 to 1.312, p=0.044), CRP (HR: 1.011, 95% CI: 1.005 to 1.016, p=0.00), Gensini score (HR: 1.013, 95% CI: 1.004 to 1.022, p=0.006) and operation strategy (HR: 3.263, 95% CI: 1.379 to 7.722, p=0.007) were found to be predictors of MACCE.

Predictors of repeat revascularization were CRP (HR: 1.011, 95% CI: 1.005 to 1.016, p=0.00), Gensini score (HR: 1.012, 95% CI: 1.002 to 1.022, p=0.018) and operation strategy (HR: 6.968, 95% CI: 2.036 to 23.842, p=0.002). There were no significant predictors of other endpoints.

Discussion

In this retrospective study, CABG was shown to be superior to PCI with 2nd-generation DESs in young patients with LM and/or three-vessel disease in terms of the incidence of MACCE, which was driven mainly by repeat revascularization. There were no significant differences the hard endpoints death, MI and stoke in line with the outcomes of the recent
EXCLE trial (8). After adjustment by propensity-score matching to minimize selection bias, the conclusion was the same as in the overall population. Although this study was limited by its observational design, this is the first report specifically addressing the issue of LM and three-vessel disease in young patients, and it evaluated the potential noninferiority of PCI over CABG. Therefore, our results would be helpful when making a clinical decision in real-world practice, especially for young CAD patients.

It is a good thing that young patients have better baseline characteristics along with fewer and milder complications, so it is essential to consider the broad indications and long-term prognosis. In our study, there were no obvious differences between the two groups in baseline characteristics, except the CABG group had more complex coronary anatomy and lower usage of dual antiplatelets. However, the PCI group showed a higher incidence of MACCE and repeat revascularization.

As in some previous studies, we found that CABG was better than PCI for the composite endpoint of MACCE and repeat revascularization both in LM(6) and three-vessel disease(3, 12, 15). One of the reasons is that after PCI, progressive atherosclerosis can lead to new, severe stenosis and plaque rupture that may cause ischemia and repeat revascularization, and CABG offers better protection by bypassing a large proportion of obstructive lesions or vulnerable plaques, minimizing the impact of progressive disease in the entire upstream proximal vessel (16). Moreover, there was more incomplete revascularization in the PCI group that needed more than one intervention operation, whereas patients achieving complete revascularization showed similar outcomes between PCI and CABG (17). In addition, more routine angiographic follow-up was performed to detect early in-stent restenosis in patients treated with PCI rather than those with CABG. Many patients with PCI receive repeat revascularization that is angiographically rather than clinically driven. Thus, the rate of repeat revascularization might be underestimated for those
patients undergoing CABG. We must recognize that with the introduction of high-pressure deployment, use of intravascular ultrasound, and improved stent design, restenosis of drug-eluting stent has diminished over time (10, 18).

Some studies proved that the PCI group had higher rates of MI (19, 20), whereas other studies (21, 22) supported our finding that there were similar rates of MI between the PCI and CABG groups. The main advantage of CABG might be the bypassing of long lesion segments by grafting, which protects, to a great extent, against target lesion MI and proximal de-novo lesion MI (10, 11). The small population and short follow-up time may be two of the reasons that caused the absence of significant differences in MI rates.

While some other studies showed that CABG resulted in significantly higher rates of stoke compared with PCI for LM or multivessel disease (5, 23), we found that the difference in rates was indistinctive. The mechanisms underlying the increased risk of stoke with CABG are likely multifactorial. First, CABG performed on-pump with cannulation and clamping of the aorta increases the rates of stoke, which may be reduced by an off-pump procedure (24). Furthermore, stoke may be less common after PCI due to the routine use of dual antiplatelets after stent implantation. However, in the present study, the CABG group also had higher usage of aspirin and clopidogrel or ticagrelor.

Partly different from Head’s study (25), we and Park et al. shared the same outcome in terms of death to a certain extent (4), i.e., that there was no significant difference in the rate of death between the PCI and CABG groups. The low mortality after treatment in both groups showed that modern revascularization techniques and adjunctive therapy can lead to excellent survival in young patients with LM and three-vessel disease. All these low incidences of MI, stoke and death might relate to the young characteristics of the patients enrolled in our study.

In the subgroup analysis, we found that CABG might lead to higher rates of MACCE in
patients who had previously suffered MI, whereas in the opposite patients, PCI caused more MACCE. No relevant studies support this discovery, so it will be important to conduct further studies to see if this finding is generalizable.

In contrast to previous reports involving multivessel or LM disease in part, we found that, along with operation strategy, the predictors of MACCE and repeat revascularization were age, CRP and Gensini score. It is possible that, the inflammation condition and coronary anatomy play an important role in the long-term curative effect, which has been verified by other studies. Kosmoidou et al. (26) found that elevated baseline CRP level was strongly associated with subsequent death, MI and stroke. Misumida et al. (27) detected that SYNTAX score 2 was correlated with mortality. However, some predictors such as diabetes mellitus (28), heart failure (29), chronic renal failure (30) and so on, which proved related to MACCE in other studies, were not included in our finding. From a clinical viewpoint, using these relevant variables that were considered potential predictors of MACCE in young patients with LM and three-vessel disease, represents a first step to implementing further preventive measures and tailored therapies.

Considering the discussion above, patients in the PCI group with a 2nd DES had higher rates of repeat revascularization, which did not translate into a higher incidence of the hard endpoints of MI, stroke and death. A recent meta-analysis and the PRECOMBAT study also supports our results (6, 31). The young age of our patients could explain this result, but it is important to select appropriate operations for long-term survival. The relative benefits of CABG versus PCI with stents in terms of outcomes are highly debated, particularly with each advancement in stent design. Now, the state-of-the-art stent is the second-generation DES, which is thinner and is coated with a more biocompatible polymer and new ‘limus’ drugs that allow less inflammation and a lower rate of restenosis compared with first-generation DESs (12, 32, 33). What’s more, with the development of
technology, some technique such as IVUS, OCT and kissing balloon may improve prognosis. The kissing balloon can reduce the risk of overall target lesion revascularization while the IVUS and OCT can be used to optimize stenting and guide procedural strategy (34, 35). PCI may also be preferred because of its improved early safety profile.

The internal mammary arteries have been widely used as conduits to the left anterior descending artery due to their long-term patency, and the advantage of CABG may be partially due to the completeness of revascularization (36). Although high long-term patency of the internal mammary artery is expected, some vein graft degeneration can be expected beyond 5 years (10). Multiple arterial grafting is associated with improved survival and a reduced requirement of reintervention compared with grafting of a single internal thoracic artery plus the saphenous vein (37). In the current era, routine use of the right internal mammary artery has not been widely adopted despite its identical histological features to the left internal mammary artery due to technical difficulties and concerns about a potential increase in rates of bleeding and wound complication (20).

Saphenous vein grafts are routinely used in CABG surgery as additional conduits to artery grafts. However, saphenous vein grafts typically present accelerated atherosclerosis resulting in a high rate of stenosis or occlusion of the graft, which contributes to higher morbidity and mortality (38). In the case of graft failure, repeat revascularization after either PCI or CABG is necessary in a certain number of patients, if appropriate.

Nevertheless, in addition to an increased operation difficulty, patients undergoing re-CABG have a 2- to 4-fold higher mortality than they do in the first operation, whereas PCI in patients previously treated with CABG is associated with worse acute and long-term outcomes compared with native artery PCI (39, 40).

Our study had some limitations. First, it was a nonrandomized, retrospective study,
although we performed propensity-score matching to minimize the potential selection bias and ascertainment bias. Second, the follow-up duration and number of enrolled patients might not be sufficient to evaluate the long-term outcomes of revascularization. Third, this was a single-center study that only included Chinese, and more ethnicities are required in further trials. Fourth, because the treatment choice was left to the physician or patients, selection bias was inevitable. Moreover, some patients who underwent CABG, had the angiography done in outside hospitals rather than our hospital, which affected our evaluation of the lesion. Finally, we used the prevalent SYNTAX score with the Gensini score to estimate the anatomic complexity due to practical considerations.

Conclusion

In our retrospective study evaluating PCI with second DESs versus CABG in young patients with LM and/or three-vessel disease in the real world, the PCI group suffered higher rates of MACCE compared with the CABG group, which was driven by repeat revascularization, but did not translate into a higher incidence of hard endpoints, such as MI, stroke and death. In our opinion, for these young patients, along with the technical development of second-generation DESs for PCI, higher use of IVUS and fractional flow reserve and new imaging techniques, such as OCT, may be an alternative treatment strategy to CABG for long-term prognosis. Of course, further research and longer follow-up durations are indispensable.

Abbreviation

BMI: body mass index; CABG: coronary artery bypass grafting; CAD: coronary artery disease; PCI: percutaneous coronary intervention; DES: drug eluting stent; CCR: creatinine clearance rate; UA: uric acid; LVEF: left ventricular ejection fraction; HF: heart failure; MI: myocardial infarction; CRP: C-reactive protein; MACCE: major adverse cardiovascular or
cerebrovascular events which are the composite of all-cause death, stroke, MI, and ischemia-driven revascularization; HT: hypertension; DM: diabetes mellitus; LM disease: left main coronary artery disease, which includes LM coronary artery disease in isolation and LM coronary artery disease with multivessel disease (three-vessel disease); IVUS: intravascular ultrasound; OCT: optical coherence tomography.

Declarations

**Ethics approval and consent to participate**

The ethics committee at our hospital (Beijing Anzhen Hospital) reviewed our study protocol and approved the use of clinical data for the study (No: 2018020X). Because of the retrospective nature of our trial, the need for patient informed consent was waived.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

XC, XZ, YY, XZ, MN, TF, ZL and QZ designed the study. XC, XZ and YY contributed the data collection. XC, YY and XZ did the data analysis. XC and MN constructed to the tables and
figures. XC, TF, ZL and QZ interpreted the data. XC wrote the first draft of the manuscript. All authors critically reviewed the manuscript and approved the final version and its submission.

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References

1. Modolo R, Chichareon P, Kogame N, Dressler O, Crowley A, Ben-Yehuda O, et al. Contemporary Outcomes Following Coronary Artery Bypass Graft Surgery for Left Main Disease. J Am Coll Cardiol. 2019;73(15):1877-86.

2. Milojevic M, Serruys PW, Sabik JF, 3rd, Kandzari DE, Schampaert E, van Boven AJ, et al. Bypass Surgery or Stenting for Left Main Coronary Artery Disease in Patients With Diabetes. J Am Coll Cardiol. 2019;73(13):1616-28.

3. Mohr FW, Morice M-C, Kappetein AP, Feldman TE, Ståhle E, Colombo A, et al. Coronary artery bypass graft surgery versus percutaneous coronary intervention in patients with three-vessel disease and left main coronary disease: 5-year follow-up of the randomised, clinical SYNTAX trial. The Lancet. 2013;381(9867):629-38.

4. Park DW, Ahn JM, Yun SC, Yoon YH, Kang DY, Lee PH, et al. 10-Year Outcomes of Stents Versus Coronary Artery Bypass Grafting for Left Main Coronary Artery Disease. J Am Coll Cardiol. 2018;72(23 Pt A):2813-22.
5. Head SJ, Milojevic M, Daemen J, Ahn JM, Boersma E, Christiansen EH, et al. Stroke Rates Following Surgical Versus Percutaneous Coronary Revascularization. J Am Coll Cardiol. 2018;72(4):386-98.

6. Ahn JM, Roh JH, Kim YH, Park DW, Yun SC, Lee PH, et al. Randomized Trial of Stents Versus Bypass Surgery for Left Main Coronary Artery Disease: 5-Year Outcomes of the PRECOMBAT Study. J Am Coll Cardiol. 2015;65(20):2198-206.

7. Neumann FJ, Sousa-Uva M, Ahlsson A, Alfonso F, Banning AP, Benedetto U, et al. 2018 ESC/EACTS Guidelines on myocardial revascularization. Eur Heart J. 2019;40(2):87-165.

8. Stone GW, Sabik JF, Serruys PW, Simonton CA, Genereux P, Puskas J, et al. Everolimus-Eluting Stents or Bypass Surgery for Left Main Coronary Artery Disease. The New England journal of medicine. 2016;375(23):2223-35.

9. Kukreja N, Serruys PW, De Bruyne B, Colombo A, Macaya C, Richardt G, et al. Sirolimus-eluting stents, bare metal stents or coronary artery bypass grafting for patients with multivessel disease including involvement of the proximal left anterior descending artery: analysis of the Arterial Revascularization Therapies study part 2 (ARTS-II). Heart. 2009;95(13):1061-6.

10. Mäkikallio T, Holm NR, Lindsay M, Spence MS, Erglis A, Menown IBA, et al. Percutaneous coronary angioplasty versus coronary artery bypass grafting in treatment of unprotected left main stenosis (NOBLE): a prospective, randomised, open-label, non-inferiority trial. The Lancet. 2016;388(10061):2743-52.

11. Milojevic M, Head SJ, Parasca CA, Serruys PW, Mohr FW, Morice MC, et al. Causes of Death Following PCI Versus CABG in Complex CAD: 5-Year Follow-Up of SYNTAX. J Am Coll Cardiol. 2016;67(1):42-55.

12. Tsuneyoshi H, Komiya T, Kadota K, Shimamoto T, Sakai J, Hiraoka T, et al. Coronary
artery bypass surgery is superior to second generation drug-eluting stents in three-vessel coronary artery disease: a propensity score matched analysis. Eur J Cardiothorac Surg. 2017;52(3):462-8.

13. Sabik JF, 3rd, Blackstone EH, Gillinov AM, Smedira NG, Lytle BW. Occurrence and risk factors for reintervention after coronary artery bypass grafting. Circulation. 2006;114(1 Suppl):I454-60.

14. Head SJ, Davierwala PM, Serruys PW, Redwood SR, Colombo A, Mack MJ, et al. Coronary artery bypass grafting vs. percutaneous coronary intervention for patients with three-vessel disease: final five-year follow-up of the SYNTAX trial. Eur Heart J. 2014;35(40):2821-30.

15. Park SJ, Ahn JM, Kim YH, Park DW, Yun SC, Lee JY, et al. Trial of everolimus-eluting stents or bypass surgery for coronary disease. The New England journal of medicine. 2015;372(13):1204-12.

16. Cui K, Lyu S, Song X, Liu H, Yuan F, Xu F, et al. Drug-Eluting Stent Versus Coronary Artery Bypass Grafting for Diabetic Patients With Multivessel and/or Left Main Coronary Artery Disease: A Meta-Analysis. Angiology. 2019:3319719839885.

17. Ahn JM, Park DW, Lee CW, Chang M, Cavalcante R, Sotomi Y, et al. Comparison of Stenting Versus Bypass Surgery According to the Completeness of Revascularization in Severe Coronary Artery Disease: Patient-Level Pooled Analysis of the SYNTAX, PRECOMBAT, and BEST Trials. JACC Cardiovasc Interv. 2017;10(14):1415-24.

18. Park SJ, Kim YH, Park DW, Lee SW, Kim WJ, Suh J, et al. Impact of intravascular ultrasound guidance on long-term mortality in stenting for unprotected left main coronary artery stenosis. Circ Cardiovasc Interv. 2009;2(3):167-77.

19. Lee CW, Ahn JM, Cavalcante R, Sotomi Y, Onuma Y, Suwannasom P, et al. Coronary Artery Bypass Surgery Versus Drug-Eluting Stent Implantation for Left Main or
Multivessel Coronary Artery Disease: A Meta-Analysis of Individual Patient Data. JACC Cardiovasc Interv. 2016;9(24):2481-9.

20. Jeong DS, Lee YT, Chung SR, Jeong JH, Kim WS, Sung K, et al. Revascularization in left main coronary artery disease: comparison of off-pump coronary artery bypass grafting vs percutaneous coronary intervention. Eur J Cardiothorac Surg. 2013;44(4):718-24.

21. Chieffo A, Meliga E, Latib A, Park SJ, Onuma Y, Capranzano P, et al. Drug-eluting stent for left main coronary artery disease. The DELTA registry: a multicenter registry evaluating percutaneous coronary intervention versus coronary artery bypass grafting for left main treatment. JACC Cardiovasc Interv. 2012;5(7):718-27.

22. Putzu A, Gallo M, Martino EA, Ferrari E, Pedrazzini G, Moccetti T, et al. Coronary artery bypass graft surgery versus percutaneous coronary intervention with drug-eluting stents for left main coronary artery disease: A meta-analysis of randomized trials. Int J Cardiol. 2017;241:142-8.

23. Palmerini T, Biondi-Zoccai G, Reggiani LB, Sangiorgi D, Alessi L, De Servi S, et al. Risk of stroke with coronary artery bypass graft surgery compared with percutaneous coronary intervention. J Am Coll Cardiol. 2012;60(9):798-805.

24. Head SJ, Borgermann J, Osnabrugge RL, Kieser TM, Falk V, Taggart DP, et al. Coronary artery bypass grafting: Part 2--optimizing outcomes and future prospects. Eur Heart J. 2013;34(37):2873-86.

25. Head SJ, Milojevic M, Daemen J, Ahn J-M, Boersma E, Christiansen EH, et al. Mortality after coronary artery bypass grafting versus percutaneous coronary intervention with stenting for coronary artery disease: a pooled analysis of individual patient data. The Lancet. 2018;391(10124):939-48.

26. Kosmidou I, Redfors B, Chen S, Crowley A, Lembo NJ, Karpaliotis D, et al. C-reactive
protein and prognosis after percutaneous coronary intervention and bypass graft surgery for left main coronary artery disease: Analysis from the EXCEL trial. Am Heart J. 2019;210:49-57.

27. Misumida N, Ahmed AE, Barlow M, Goodwin R, Goodwin E, Musa A, et al. Prognostic Value of Anatomical SYNTAX Score and SYNTAX Score II in Veterans With Left Main and/or Three-Vessel Coronary Artery Disease. Am J Cardiol. 2018;122(2):213-9.

28. Roffi M, Angiolillo DJ, Kappetein AP. Current concepts on coronary revascularization in diabetic patients. Eur Heart J. 2011;32(22):2748-57.

29. Kang SH, Ahn JM, Lee CH, Lee PH, Kang SJ, Lee SW, et al. Differential Event Rates and Independent Predictors of Long-Term Major Cardiovascular Events and Death in 5795 Patients With Unprotected Left Main Coronary Artery Disease Treated With Stents, Bypass Surgery, or Medication: Insights From a Large International Multicenter Registry. Circ Cardiovasc Interv. 2017;10(7).

30. Cooper WA, O'Brien SM, Thourani VH, Guyton RA, Bridges CR, Szczech LA, et al. Impact of renal dysfunction on outcomes of coronary artery bypass surgery: results from the Society of Thoracic Surgeons National Adult Cardiac Database. Circulation. 2006;113(8):1063-70.

31. Shah R, Morsy MS, Weiman DS, Vetrovec GW. Meta-Analysis Comparing Coronary Artery Bypass Grafting to Drug-Eluting Stents and to Medical Therapy Alone for Left Main Coronary Artery Disease. Am J Cardiol. 2017;120(1):63-8.

32. Sarno G, Lagerqvist B, Nilsson J, Frobert O, Hambraeus K, Varenhorst C, et al. Stent thrombosis in new-generation drug-eluting stents in patients with STEMI undergoing primary PCI: a report from SCAAR. J Am Coll Cardiol. 2014;64(1):16-24.

33. Deb S, Wijeysundera HC, Ko DT, Tsubota H, Hill S, Fremes SE. Coronary artery bypass graft surgery vs percutaneous interventions in coronary revascularization: a
systematic review. JAMA. 2013;310(19):2086-95.

34. D’Ascenzo F, Omede P, De Filippo O, Cerrato E, Autelli M, Trabattoni D, et al. Impact of Final Kissing Balloon and of Imaging on Patients Treated on Unprotected Left Main Coronary Artery With Thin-Strut Stents (From the RAIN-CARDIOGROUP VII Study). The American journal of cardiology. 2019;123(10):1610-9.

35. Koskinas KC, Nakamura M, Raber L, Colleran R, Kadota K, Capodanno D, et al. Current use of intracoronary imaging in interventional practice - Results of a European Association of Percutaneous Cardiovascular Interventions (EAPCI) and Japanese Association of Cardiovascular Interventions and Therapeutics (CVIT) Clinical Practice Survey. EuroIntervention. 2018;14(4):e475-e84.

36. Farooq V, Serruys PW, Garcia-Garcia HM, Zhang Y, Bourantas CV, Holmes DR, et al. The negative impact of incomplete angiographic revascularization on clinical outcomes and its association with total occlusions: the SYNTAX (Synergy Between Percutaneous Coronary Intervention with Taxus and Cardiac Surgery) trial. J Am Coll Cardiol. 2013;61(3):282-94.

37. Kieser TM, Lewin AM, Graham MM, Martin BJ, Galbraith PD, Rabi DM, et al. Outcomes associated with bilateral internal thoracic artery grafting: the importance of age. Ann Thorac Surg. 2011;92(4):1269-75; discussion 75-6.

38. Scarsini R, Zivelonghi C, Pesarini G, Vassanelli C, Ribichini FL. Repeat revascularization: Percutaneous coronary intervention after coronary artery bypass graft surgery. Cardiovasc Revasc Med. 2016;17(4):272-8.

39. Sabik JF, 3rd, Blackstone EH, Houghtaling PL, Walts PA, Lytle BW. Is reoperation still a risk factor in coronary artery bypass surgery? Ann Thorac Surg. 2005;80(5):1719-27.

40. Tejada JG, Velazquez M, Hernandez F, Albarran A, Gomez I, Rodriguez S, et al.
Percutaneous revascularization in patients with previous coronary artery bypass graft surgery. Immediate and 1-year clinical outcomes. Int J Cardiol. 2009;134(2):201-6.

Tables

Table 1: Baseline characteristic of overall patients

|                          | CABG n= 81 | PCI with 2nd DES n= 132 | P value |
|--------------------------|------------|-------------------------|---------|
| Age (year)               | 42.15±2.82 | 41.67±3.49              | 0.295   |
| Male                     | 7390.1%    | 12493.9%                | 0.305   |
| Smoking (current or former) | 46 56.8%   | 96 72.7%                | 0.017   |
| Drinking (current or former) | 17 (21%)   | 38 (28.8%)              | 0.207   |
| Hypertension             | 43 (53.1%) | 80 60.6%                | 0.281   |
| Diabetes mellitus        | 21 (25.9%) | 36 (27.3%)              | 0.829   |
| Hyperlipidemia           | 7 (8.6%)   | 29 (22.0%)              | 0.012   |
| BMI (kg/m²)              | 26.80±3.18 | 28.05±3.75              | 0.013   |
| CCR (ml/min)             | 121.34±27.41 | 130.00±32.08         | 0.045   |
| UA (umol/l)              | 347.56±97.86 | 390.03±90.05         | 0.001   |
| CRP (mg/l)               | 2.59±5.44  | 6.32±38.02              | 0.383   |
| Family history           | 16 (19.8%) | 39 (29.5%)              | 0.113   |
| Blood group              |            |                        |         |
| A                        | 27 (33.3%) | 43 (32.6%)              | 0.962   |
|                          | 22 (27.2%) | 37 (28.0%)              |         |
|                      | Group 1 | Group 2 | p-value |
|----------------------|---------|---------|---------|
| **B**                | 9 (11.1%) | 12 (9.1%) |         |
| **AB**               | 23 (28.4%) | 40 (30.3%) |         |
| **O**                |          |         |         |
| **LVEF(%)**          | 59.95±8.41 | 61.83±7.25 | 0.085   |
| **Prior HF**         | 17 (21.0%) | 38 (28.8%) | 0.207   |
| **Prior MI**         | 14 (17.3%) | 31 (23.5%) | 0.282   |
| **Prior stroke**     | 1 (1.2%) | 3 (2.3%) | 0.664   |
| **Prior stent**      | 6 (7.4%) | 16 (12.1%) | 0.272   |
| **Collateral circulation** | 3 (3.7%) | 10 (7.6%) | 0.378   |
| **Lesion type**      |          |         | 0.366   |
| LM with/without three-vessel disease | 12 (14.8%) | 26 (19.7%) |         |
| Three-vessel disease isolated | 69 (85.2%) | 106 (80.3%) |         |
| **Gensini score**    | 86.64±36.77 | 69.80±35.10 | 0.001   |
| <60                  | 25 (30.9%) | 62 (47.0%) |         |
| ≥60                  | 56 (69.1%) | 70 (53.0%) |         |
| **Discharge medication** |       |         |         |
| Aspirin              | 80 (98.8%) | 131 (99.2%) | 1.00    |
| P₇₂₃ inhibitors      | 74 (91.4%) | 131 (99.2%) | 0.005   |
Table 2. Comparison of traditional echocardiographic parameters between control and MHD groups

| Parameter     | MHD                 | Normal              | P-value |
|---------------|---------------------|---------------------|---------|
| LVIDD (mm)    | 52.56±6.13          | 46.06±3.07          | <0.001  |
| LVIDS (mm)    | 36.65±5.55          | 31.11±2.84          | <0.001  |
| IVST (mm)     | 11.56±1.62          | 8.86±1.38           | <0.001  |
| LVPWT (mm)    | 11.24±1.50          | 8.14±1.38           | <0.001  |
| LAD (mm)      | 42.38±5.29          | 34.86±3.12          | <0.001  |
| LVEF (%)      | 58.24±5.16          | 60.11±4.00          | 0.095   |
| LVMI (g/m²)   | 56.59±9.11          | 31.60±3.82          | <0.001  |
| E/A           | 0.99±0.15           | 1.15±0.19           | <0.001  |
| E/e'          | 7.97±1.67           | 7.08±1.36           | 0.019   |

Table 3: Baseline characteristic of the patients after adjusted by propensity-score matching

|                  | CABG n= 46 | PCI with 2nd DES n= 46 | P value |
|------------------|------------|------------------------|---------|
| Age (year)       | 42.04 ±3.00| 42.02 ±2.56            | 0.970   |
| Male             | 4291.3%    | 4393.5%                | 0.694   |

BMI: Body mass index; CABG: coronary artery bypass grafting; CCR: creatinine clearance rate; CRP: C-reactive protein; DES: drug eluting stent; HF: heart failure; LM: left main; LVEF: left ventricular ejection fraction; MI: myocardial infarction; PCI: percutaneous coronary intervention; UA: uric acid

Data are expressed as mean ± SD. Bold number: P < 0.05

HD: hemodialysis; BMI: body mass index; BP: systolic blood pressure; DBP: diastolic blood pressure; CREA: serum creatinine; ESRD: end-stage renal disease.
| Condition                     | Group A   | Group B   | p-value |
|-------------------------------|-----------|-----------|---------|
| Smoking (current or former)   | 29 (63.0%)| 28 (60.9%)| 0.830   |
| Drinking (current or former)  | 13 (28.3%)| 12 (26.1%)| 0.815   |
| Hypertension                  | 23 (50.0%)| 26 (56.5%)| 0.531   |
| Diabetes mellitus             | 12 (26.1%)| 10 (21.7%)| 0.625   |
| Hyperlipidemia                | 5 (10.9%)  | 6 (13.0%)  | 0.748   |
| BMI (kg/m²)                   | 27.46 ±3.23| 27.58±3.56| 0.873   |
| CCR (ml/min)                  | 122.34±27.49| 122.77±33.58| 0.947   |
| UA (umol/l)                   | 381.33±82.28| 385.45±84.74| 0.813   |
| CRP (mg/l)                    | 2.59±5.44  | 6.32±38.02 | 0.935   |
| Family history                | 10 (21.7%) | 12 (26.1%) | 0.625   |
| Blood group                   |           |           | 0.850   |
| A                             | 17 (37.0%) | 16 (34.8%) |         |
| B                             | 13 (28.3%) | 13 (28.3%) |         |
| AB                            | 6 (13.0%)  | 4 (8.7%)   |         |
| O                             | 10 (21.7%) | 13 (28.3%) |         |
| LVEF(%)                       | 61.59±8.00 | 59.72±7.70 | 0.256   |
| Prior HF                      | 8 (17.4%)  | 10 (21.7%) | 0.599   |
| Prior MI                      | 10 (21.7%) | 11(23.9%)  | 0.804   |
| Category                                      | Group 1 | Group 2 | p-value |
|----------------------------------------------|---------|---------|---------|
| Prior stroke                                 | 1 (2.2%) | 1 (2.2%) | 1.000   |
| Prior stent                                  | 4 (8.7%) | 1 (2.2%) | 0.361   |
| Collateral circulation                       | 3 (6.5%) | 4 (8.7%) | 0.694   |
| Lesion type                                  |         |         | 0.562   |
| LM with/without three-vessel disease         | 8 (17.4%) | 4 (13.0%) |         |
| Three-vessel disease isolated                | 38 (82.6%) | 40 (87.0%) |         |
| Gensini score                                | 74.85±28.06 | 78.75±38.66 | 0.581   |
| Gensini score <60                            | 19 (41.30%) | 17 (37.0%) |         |
| Gensini score ≥60                            | 27 (58.70%) | 29 (63.0%) |         |
| Discharge medication                         |         |         |         |
| Aspirin                                      | 46 (100%) | 46 (100%) | 1.000   |
| P$_2$Y$_{12}$ inhibitor                      | 44 (95.7%) | 46 (100%) | 0.495   |
| Dual antiplatelet                            | 44 (95.7%) | 46 (100%) | 0.495   |
| Statin                                       | 46 (100%) | 45 (97.8%) | 1.000   |
| Beta blocker                                 | 39 (84.8%) | 42 (91.3%) | 0.522   |

**BMI**: body mass index; **CABG**: coronary artery bypass grafting; **CCR**: creatinine clearance rate; **CRP**: C-reactive protein; **DES**: drug eluting stent; **HF**: heart failure; **LM**: left main; **LVEF**: left ventricular ejection fraction; **MI**: myocardial infarction; **PCI**: percutaneous coronary intervention; **UA**: uric acid
### Table 4: Clinical outcome after adjusted by propensity-score match

|                     | PCI (n=46) | CABG (n=46) | HR (95% CI)       | P value |
|---------------------|------------|-------------|-------------------|---------|
| MACCE               | 15 (32.6%) | 5 (10.9%)   | 4.496 (1.592, 12.695) | 0.005   |
| MI                  | 1 (2.2%)   | 1 (2.2%)    | 2635462740 (0.00, 2.092E+040) | 0.550   |
| Stoke               | 0          | 2 (4.3%)    | 0 (0.00, 5.141E+16)   | 0.658   |
| Death               | 0          | 1 (2.2%)    | 0.001 (0.00, 1.299E+036) | 0.876   |
| Repeat revascularization | 14 (30.4%) | 2 (4.3%)  | 11.66 (2.449, 55.51)   | 0.002   |

CABG: coronary artery bypass grafting; CI: confidence interval; HR: hazard ratio; MACCE: major adverse cardiovascular or cerebrovascular events which is the composite of all-cause death, stroke, MI, or repeat revascularization; MI: myocardial infarction; PCI: percutaneous coronary intervention.

**Additional Files**

Additional file 1.xls: Table 2: Clinical outcome of overall patients before adjusted by propensity-score matching.

CABG: coronary artery bypass grafting; CI: confidence interval; HR: hazard ratio; MACCE: major adverse cardiovascular or cerebrovascular events which is the composite of all-cause death, stroke, MI, or repeat revascularization; MI: myocardial infarction; PCI: percutaneous coronary intervention.

Additional file 2.tif: Standardized differences before and after propensity-score matching.

An absolute standardized difference of less than 10% indicates a good match.

Abbreviations as in Table 1.

**Figures**
Figure 1

Flow chart of enrolled patients PCI: percutaneous coronary intervention; CABG: coronary artery bypass grafting; STEMI: ST-segment-elevation myocardial infarction; NSTEMI: non-ST-segment-elevation myocardial infarction; LM disease: left main coronary artery disease
Figure 2

Kaplan-Meier cumulative event curves of MACCE and secondary end points before propensity-score matching. The adjusted risk of PCI relative to CABG is shown. PCI: percutaneous coronary intervention; CABG: coronary artery bypass graft; MI: myocardial infarction
Figure 3

Kaplan-Meier cumulative event curves of MACCE and secondary end points after propensity-score matching. The adjusted risk of PCI relative to CABG is shown.

PCI: percutaneous coronary intervention; CABG: coronary artery bypass graft; MI: myocardial infarction
| Subgroup | Events/total number | HR (95% CI) | p for interaction |
|----------|---------------------|-------------|------------------|
| **Sex**  |                     |             |                  |
| Women    | 2/16                | *11899.479 (0.005-30715362593) | 0.919            |
| Men      | 32/107              | 2.928 (1.203-7.128)             |                  |
| **HT**   |                     |             |                  |
| Yes      | 21/123              | 8.41 (1.829-38.678)             | 0.209            |
| No       | 13/90               | 1.325 (0.357-4.914)             |                  |
| **DM**   |                     |             |                  |
| Yes      | 9/57                | 2.461 (0.371-16.315)            | 0.145            |
| No       | 25/156              | 4.577 (1.476-14.192)            |                  |
| **Prior MI** |                 |             |                  |
| Yes      | 7/45                | 0.652 (0.125-3.397)             | 0.031            |
| No       | 27/168              | 6.048 (1.194-18.344)            |                  |
| **Prior HF** |                 |             |                  |
| Yes      | 12/55               | 0.926 (0.226-3.785)             | 0.115            |
| No       | 22/158              | 6.672 (1.934-23.642)            |                  |
| **LM disease** |               |             |                  |
| Yes      | 7/38                | 2.695 (0.246-29.591)            | 0.839            |
| No       | 27/175              | 3.409 (1.268-9.166)             |                  |
| **Gensini Score** |         |             |                  |
| <60      | 13/87               | 2.139 (0.444-10.302)            | 0.764            |
| >=60     | 21/126              | 2.791 (0.973-8.009)             |                  |

**Figure 4**

Subgroup analysis based on sex, HT, DM, prior MI, prior HF, LM disease and Gensini score HT: hypertension; DM: diabetes mellitus; MI: myocardial infarction; HF: heart failure; LM disease: left main coronary artery disease, which includes LM coronary artery disease in isolation and LM coronary artery disease with multivessel disease (three-vessel disease); []: Because the data point is outside the axis limits.

**Supplementary Files**

This is a list of supplementary files associated with the primary manuscript. Click to download.

Additional file 1.xls
Additional file 2.tif