

STUDY PROTOCOL

Anti-inflammatory therapy during percutaneous coronary intervention

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I. Introduction

1. Purpose of the study

We hypothesize that colchicine may effectively reduce inflammation and peri-procedural MI in patients not acutely loaded with high-dose statin prior to PCI. We further hypothesize that colchicine's effects would be additive in patients undergoing PCI on a background of acute high-dose statin pre-treatment. We aim to determine the effects of colchicine 1.2mg administered 1 to 2 hours prior to coronary angiography, followed by a 0.6mg dose 1 hour later, on post-procedural markers of inflammation and cardiac biomarkers.

2. Background and Significance

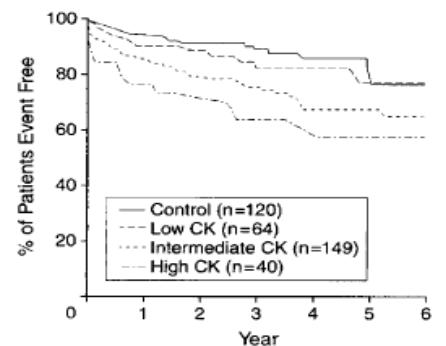
ACS and PCI are inflammatory events. Inflammatory cells in coronary plaque are increased in patients with ACS, along with a consequent increase in systemic inflammatory markers. Shu et al observed significantly higher levels of interleukin-6 (IL-6), high-sensitivity C-reactive protein (CRP) and matrix metalloproteinase-9 (MMP-9) in patients with ACS versus stable angina. Furthermore, markers were significantly more elevated in patients with stable angina than in normal controls (1).

PCI further induces an inflammatory response by disrupting coronary plaque and coronary artery endothelium during balloon inflations. Robertson et al noted increases of matrix MMP-9 and IL-6 locally in the culprit coronary artery of ACS patients during PCI, along with a systemic inflammatory response (2). In one study evaluating 25 patients predominantly with ACS undergoing PCI, IL-6 increased as early as 1 hour after, compared with before, PCI (3). Sardella et al observed a significantly greater level of cell-associated beta2-integrin, a subunit that plays a role in leukocyte recruitment and adhesion at sites of endothelial injury, in ACS patients versus age-matched healthy volunteers, with a significant further increase detected immediately after balloon deflation during primary PCI (4). The inflammatory response to PCI has also been studied in stable CAD using various markers including pentraxin-3, chemokines CCL2 and CXCL16, CRP, VACM-1, and IL-6 (5-6). One study suggests that uncomplicated diagnostic coronary angiography may in itself be sufficient to trigger a systemic inflammatory response in patients with stable CAD (6).

The inflammatory state at the time of PCI predicts clinical outcomes. Kwaijtaal et al demonstrated the prognostic value of IL-6, CRP, and IL-10 measured 6 weeks after PCI in predicting late cardiac events (relative risk 3.9, 2.5, and 2.5, respectively) (7). Chan et al further demonstrated that rates of peri-procedural MI increase with pre-procedural CRP (8), and high levels of CRP have been associated with adverse outcomes after PCI (8-10). Interleukin-1 (IL-1) is another inflammatory cytokine involved in tissue inflammation and ischemia reperfusion injury, while IL-1 receptor antagonist competitively blocks the binding of IL-1 to its receptor (11-12) and correlates with severity of inflammation even when IL-1 levels are low (13). Patti et al demonstrated circulating IL-1 receptor antagonist to be an independent predictor of cardiac events in an unselected group of patients with symptomatic CAD undergoing PCI (14). Lastly, pentraxin-3, produced at sites of inflammation and stored in neutrophil specific granules to be readily released upon activation, is a strong predictor of late lumen loss after PCI (15-17).

Rates of peri-procedural MI are significantly decreased in patients with CAD undergoing PCI who are pre-treated with statins. The ARMYDA (Atorvastatin for Reduction of Myocardial Damage During Angioplasty) study demonstrated decreased peri-procedural MI (5% versus 18% in the placebo group, 72% relative risk reduction) with atorvastatin pre-treatment (40mg for 7 days) prior to elective PCI in statin-naïve patients with stable CAD (18). The NAPLES (Novel Approaches for Preventing or Limiting Events) II study further demonstrated decreased rates of peri-procedural MI (9.5% versus 15.8% in the control group, 40% relative risk reduction) with atorvastatin pretreatment (80mg 24 hours prior to PCI) in the same patient population (19). A similar benefit was noted in the ACS population with high-dose statin pretreatment a mean of 12 hours prior to PCI and an additional dose 2 hours prior to PCI in the ARMYDA-ACS study (30-day incidence of major adverse cardiac events 5% versus 17% in the placebo group) (20).

Elevation of cardiac enzymes following PCI occurs in 6% to 34% of patients (21-26), with incremental increases in subsequent death and MI noted with incremental increase in post-PCI cardiac biomarkers (see figure) (22). The beneficial effects of statins prior to PCI are likely to be independent of their lipid-lowering actions, given that significant changes in lipid levels take days to weeks. One proposed mechanism of benefit of peri-procedural statin use is the attenuation of the pro-inflammatory state in ACS and PCI (27-28). The anti-inflammatory effects of statins induce plaque stabilization leading to less micro-embolization and side-branch occlusion during PCI, thereby decreasing peri-procedural myocardial necrosis.

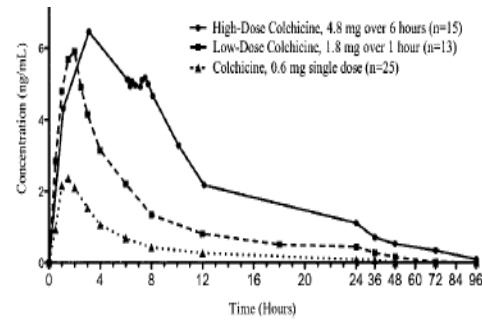


Despite the benefits statins offer, up to 40% of patients are not on statin-therapy prior to PCI (29). Statins are often withheld in patients with elevated liver enzymes, not an uncommon finding in the high-risk ACS population. In a large meta-analysis, statin therapy increased the risk of adverse events by 39% (odds ratio of 1.4) when compared with placebo (30). In this analysis, adverse events such as myalgia and liver function elevations were responsible for almost two-thirds of the adverse events reported, and, although these events are not urgent, they are enough to account for the gap in statin therapy in the population undergoing PCI. Furthermore, statins must also be given 12 to 24 hours before PCI to take effect (probably due to the kinetics of inhibiting post-translational modification of the small G proteins Rho and Ras), which limits the number of patients for whom statins are a beneficial option.

Given the significant number of patients not on statin therapy prior to PCI (29), and the apparent benefit of anti-inflammatory therapy in patients undergoing PCI, there is a need for a more rapid acting anti-inflammatory agent, especially prior to urgent PCI in high-risk ACS cases. Colchicine is a treatment of choice in gout and familial Mediterranean fever due to its rapid anti-inflammatory effect via inhibition of neutrophils and other inflammatory cells. The anti-inflammatory effects of colchicine are well studied, albeit incompletely elucidated (31-34). Our group demonstrated colchicine's interference with neutrophil-endothelial interactions by eliminating the E-selectin-mediated increment in endothelial adhesiveness to neutrophils in response to IL-1 and tumor necrosis factor- α

in-vitro within 4 hours of administering low, prophylactic, concentrations of colchicine (35). At higher, therapeutic, concentrations colchicine also diminished the expression of L-selectin on the surface of neutrophils (detectable after 1 hour) (35). Terkeltaub et al demonstrated the pharmacokinetic properties, as well as the superior safety profile without loss of efficacy of the low-dose colchicine regimen (1.2mg followed by 0.6mg 1 hour later) that is now standard of care in the treatment of gout (36). The adverse event profile was similar to that of the placebo group, with 23% of patients having diarrhea and none having severe diarrhea or vomiting (36). We have recently demonstrated that in gout patients, the use of colchicine was associated with a $\geq 50\%$ decrease in MI rates in a cohort of VA patients (37).

Only one study has evaluated the use of colchicine in the CAD population (38), and no study to date has examined the use of colchicine in the PCI population. Nidorf et al showed that low-dose colchicine significantly decreased CRP levels (relative decrease of 60% compared to 11% in the no treatment group) in patients with stable CAD already on aspirin and high-dose atorvastatin therapy (38). Due to its rapidity of action (peak concentrations in 1 to 2 hours, see figure) and excellent safety profile, acute administration of colchicine may play a promising role in improving outcomes post-PCI.



When choosing markers to measure in the current proposal, rapidity of change and predictors of clinical outcomes in the setting of PCI was taken into account. CRP is the most extensively studied inflammatory marker in patients with CAD, and pre-procedural CRP is associated with peri-procedural MI (8). However, CRP is produced in the liver in response to interleukin-6 and other inflammatory cytokines, thereby accounting for the need for 24 hours before any change in CRP is noted. Interleukin-6 is not only the primary inducer of CRP, but also a marker that increases as early as 1 hour after PCI (3) and a strong predictor of late cardiac events along with CRP (7), making it a prime candidate for the primary endpoint in the current proposal.

Animal studies have shown statin therapy to reduce infarct size when given less than 3 days prior to ischemia/reperfusion but not when administered 1 to 2 weeks before ischemia/reperfusion, suggesting that the cardioprotective effect of statin therapy decreases over time (39). However, the same paper also showed that the cardioprotective effect can be restored with acute high dose statin therapy, due to modulation of the PI3K/Akt pathway (39). With chronic statin therapy there is upregulation of the phosphatase and tensin homolog, a potent inhibitor of the phosphatidyl inositol-3 kinase (PI3K)/serine/threonine kinase (Akt) pathways. However, when given acutely, statins directly *enhance* the PI3K/Akt pathways, resulting in endothelial nitric oxide synthase 3 and activation of a signal transduction cascade that leads to cardioprotective effects (40). The ARMYDA-Recapture study demonstrated benefit with a reload of high-dose statin 12 hours prior to PCI in patients with stable angina or non-ST segment elevation MI on chronic (>30 days) statin therapy (2.4-fold reduction in periprocedural MI) (41). These studies provide us with the rationale to stratify the randomization process by patients who do or do not receive a load with high-dose statin therapy 24 hours to 3 days prior to PCI,

thereby attempting to control the confounding cardioprotective effects exerted by acute high-dose statins.

3. Study Design

Prospective, randomized, double-blinded, placebo-controlled trial of patients undergoing coronary angiography in the Manhattan Veterans Affairs Hospital (primary site is Manhattan Veterans Affairs Hospital), Bellevue Hospital Center, and New York University (NYU) Langone Medical Center cardiac catheterization laboratories. Patients will be stratified into one of two groups:

- 1) Patients who received a load with high-dose statin therapy (an increase in the patient's maintenance regimen or started newly on a statin) 24 hours to 7 days prior to the procedure and continued on a daily regimen
- 2) Patients who did not receive a load with high-dose statin therapy 24 hours to 7 days prior to the procedure

***The decision to load with high-dose statin therapy prior to PCI is made clinically by the treating physician.**

Patients will then be randomized to one of two groups:

- 1) Colchicine 1.2mg 1 to 2 hours before coronary angiography, followed by colchicine 0.6mg 1 hour later
- 2) Placebo at the same time points above

II. Subject Population and Selection

1. Patient Population

Patients referred for coronary angiography at Manhattan Veterans Affairs Hospital (primary site is Manhattan Veterans Affairs Hospital), Bellevue Hospital Center, and NYU Langone Medical Center,

2. Gender of Subjects

Subjects will include both men and women. Every effort will be made to include equitable numbers of each gender.

3. Age of Subjects

Subjects more than 18 years of age will be eligible to participate in the study. The research in question applies to the entire adult population and therefore all adults may participate.

4. Racial and Ethnic Origin

Subjects of any racial or ethnic background may participate in the study. There will be no enrollment restrictions based on race or ethnic origin.

5. Inclusion Criteria

Patients must be more than 18 years of age and referred for coronary angiography.

6. Exclusion Criteria

Patients will be excluded if they meet one of the following criteria: 1) Plan for diagnostic-only coronary angiography, 2) On colchicine chronically, 3) History of intolerance to colchicine, 4) Glomerular filtration rate <30mL/minute or on dialysis, 5) Active malignancy or infection, 6) History of myelodysplasia, 7) High-dose statin load <24 hours prior to procedure, 8) Use of oral steroids or non-steroidal anti-inflammatory agents other than aspirin within 72 hours or 3 times the agent's half-life (whichever is longer), 9) Use of strong CYP3A4/P-glycoprotein inhibitors (specifically ritonavir, ketoconazole, clarithromycin, cyclosporine, diltiazem and verapamil), 10) Unable to consent, 11) Participating in a competing study, 12) Pregnant women, or 13) Patients requiring emergent cardiac catheterization.

7. Vulnerable Subjects

The study will not include vulnerable subjects as defined by current regulations.

III. Methods and Procedures

1. Methods and Procedures

Subjects for study participation will be screened from the population referred for coronary angiography at the New York University Medical Center and Bellevue Hospital and excluded if they meet any one of the following criteria: 1) planned for diagnostic-only coronary angiography, 2) on colchicine chronically, 3) history of intolerance or allergy to colchicine, 4) glomelular filtration rate <30mL/minute or on dialysis, 5) active malignancy, 6) history of myelodysplasia, 7) high-dose statin load <12 hours prior to procedure, 8) use of oral steroids or any non-steroid anti-inflammatory agent other than aspirin within 72 hours or 3 times the agent's half-life (whichever is longer), 9) Use of strong CYP3A4/P-glycoprotein inhibitors (specifically ritonavir, ketoconazole, clarithromycin, cyclosporine, diltiazem and verapamil), 10) unable to consent, 11) participating in a competing study, 12) pregnant women, or 13) any condition (e.g., psychiatric illness) or situation that, in the investigator's opinion, may put the subject at significant risk, may confound the study results, or may interfere significantly with the subject's ability to adhere with study procedures.

A. Consent and Randomization Recruitment will be conducted in accordance of the policies of the Manhattan VA Hospital, Bellevue Hospital, and NYU IRB and Federal guidelines. The attending interventional cardiology physician of all potential subjects will be contacted to determine if the subject is willing to be approached about the study. Informed consent will be sought and documented from all subjects at the earliest time possible, or at least 2 hours before coronary angiography. The rationale, procedures and potential risks of the procedures in the study will be explained to each participant by the Principal Investigator or his appointed designee. Each subject will be told that participation in the studies described in this proposal is strictly voluntary, that refusal to participate will not alter the patient's relationship with their physician, that the studies constitute research and that the information obtained will not be specifically helpful to the individual patient's care. After the subject has read the consent form, comprehension of the key elements of the study procedures and risks will be tested with verbal questions

of the consent form content. If the subject is willing to participate, the subject will sign the IRB-approved informed consent form.

A randomization code will be generated by an independent statistician using random block sizes and held by the research pharmacist. Study drug and a unique subject identifier linked to group assignment will be allocated by the research pharmacist. However, the research pharmacist will not release group assignment to the investigative team until after all subjects have completed the protocol and the database has been locked.

B. Study Technique

Patients will then be randomized to one of two groups:

- 1) Colchicine 1.2mg 1 to 2 hours before coronary angiography, followed by colchicine 0.6mg 1 hour later.
- 2) Placebo at the same time points above

Adjunctive pharmacotherapy:

- 1) All subjects will be on aspirin therapy as follows: 1) Maintenance therapy (defined as ≥ 2 days of 81 mg or 325 mg doses), *or* 2) aspirin 325 mg load ≥ 2 hours pre-baseline (with daily dose thereafter if load ≥ 1 day pre-procedure).
- 2) This is a real-world all-comers study, and there is a substantial subset of subjects referred for possible PCI who are not on maintenance clopidogrel therapy and receive a clopidogrel loading dose < 6 hours pre-procedure, including the majority of subjects who present with ACS. Therefore, it is appropriate and clinically relevant to include all subjects, regardless of timing of clopidogrel administration, as long as it is administered pre-procedure. Timing and dose of clopidogrel administration will be noted in detail. Secondary analyses will be conducted separately in a subgroup adequately loaded with clopidogrel to achieve steady-state levels pre-procedure, and a subgroup not adequately loaded with clopidogrel pre-procedure (see definition below). For studies affected by background antiplatelet therapy (i.e. neutrophil-platelet aggregation), subjects will have to meet the definition of clopidogrel therapy as follows: 1) Maintenance therapy (≥ 7 days of 75 mg), *or* 2) clopidogrel 600 mg ≥ 6 hours pre-baseline (with 75mg daily thereafter if load ≥ 1 day pre-procedure), *or* 3) clopidogrel 300 mg ≥ 12 hours pre-baseline (with 75 mg daily thereafter if load ≥ 1 day pre-procedure).
- 3) Bivalirudin therapy for a targeted activated clotting time of > 250 seconds prior to PCI

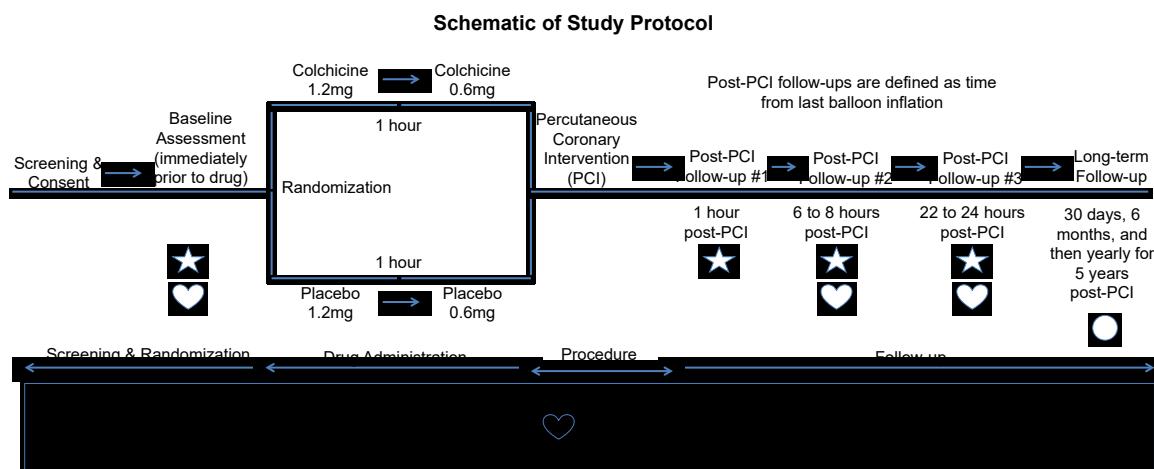
Measurement of inflammatory markers via standard venopuncture:

- 1) Immediately prior to colchicine or placebo administration as baseline
- 2) At end of procedure (30 minutes to 1 hour post-procedure)
- 3) If successful PCI is performed, 6 to 8 hours post-PCI, at the time of routine clinical blood collection
- 4) If successful PCI is performed, next day morning collection (22 to 24 hours), at the time of routine clinical blood collection

Approximately, 10cc of blood will be collected in ethylenediaminetetraacetic (EDTA) acid or sodium citrate tubes at each time point and stored immediately on ice. Approximately 5cc of blood will be prepared using a fluorescence activated cell sorter to allow for measurement of cell-associated inflammatory markers. Approximately 3cc of blood will be separated within 30 minutes by centrifugation at 4°C and 1600g for 10min, and samples will be stored at -80 °C until analysis and thawed only once. The remaining 2cc of blood will be used to measure white blood cell count and neutrophil count using a hematology analyzer. Samples will be labeled with patient unique identifier research number assigned at randomization and will only be used for the purpose of this study (no blood samples will be stored for other research studies in the future). Since baseline blood samples will be obtained from non-PCI patients due to the lack of a priori knowledge of which patients will have PCI and which will be diagnostic only, baseline and post-angiography samples will also be analyzed to test an exploratory hypothesis that colchicine also reduces markers of inflammation induced by coronary angiography/dye administration alone, albeit to a lesser degree.

Measurement of post-PCI cardiac biomarkers:

Troponin and creatine kinase-MB are measured at 6 to 8 hours and at 22 to 24 hours post-PCI via standard venopuncture as part of standard clinical care



Of note, the cardiac catheterization and PCI procedures are part of standard clinical care, along with post-PCI measurements of Troponin and CKMB. Measures of inflammatory markers in the blood and administration of colchicine versus placebo are the only research-related interventions.

C. Data Collection The following baseline variables will be prospectively collected:

Baseline Variables:

- Demographics, Height, Weight, Body mass index, Abdominal circumference (race-ethnicity self-reported, other variables measured)
- Medical history: Previous MI, previous PCI, previous coronary artery bypass surgery, hypertension, hypercholesterolemia, diabetes mellitus, peripheral vascular disease, previous stroke or transient ischemic attack, carotid artery disease (>50% stenosis), hepatitis C, and tobacco use (collected from medical records and confirmed by patient)

- Medications: statin (type and dose), aspirin, thienopyridine, cilostazol, dipyridamole, beta blocker, calcium channel blocker, nitrate, ranolazine, hydralazine, ACE inhibitor, angiotensin receptor blocker, statin, fibrate, niacin, ezetimibe, diuretic, aldosterone receptor blocker, digoxin, antiarrhythmic agent, unfractionated heparin, low molecular weight heparin within 12 hours of procedure, insulin, protease inhibitor (collected from medical records and confirmed by patient)
- Laboratory data: BUN, creatinine, glomerular filtration rate, glucose, white blood cell count, hemoglobin, total cholesterol, high density lipoprotein, low density lipoprotein, triglyceride, hemoglobin A1c, ejection fraction (normal, mild to moderately reduced, or severely reduced left ventricular systolic function) (collected from medical records)

Procedural data:

- Time of baseline blood draw and drug administration (recorded in real time)
- Indication for coronary angiography (determined by research team according to data provided in medical records)
- Time of procedural access and type of arterial access (femoral or radial) (collected from catheterization report)
- Type of contrast agent used (Hexabrix, Visipaque, Isovue) (collected from catheterization report)
- Number of native and graft vessels with greater than 70% stenosis (collected from catheterization report)
- Synergy between PCI with TAXUS and Cardiac Surgery (SYNTAX) score is a scoring system that provides information on atherosclerosis burden in patients undergoing revascularization (calculated by at least two cardiologists trained in SYNTAX scores and grouped as ≤ 17 (low), 17-32 (intermediate), or ≥ 33 (high) scores).
- If PCI performed (collected from operator questionnaire administered in real time and catheterization report):
 - Segment treated (left main, left anterior descending, left circumflex, right coronary artery, saphenous vein graft, arterial graft) and single vessel or multivessel intervention
 - Single vessel or multivessel intervention
 - Percent diameter stenosis pre- and post-PCI on semi-quantitative coronary angiography
 - Type of lesion treated (de novo or restenotic, chronic total occlusion, bifurcation lesion, calcification, visible thrombus, lesion length ≥ 33 mm), lesion site (ostial, proximal, mid-vessel, distal), and number of lesions treated

- Type of intervention with devices used (balloon only, balloon plus stent, cutting balloon, thrombectomy device, rotational atherectomy device, chronic total occlusion crossing device, filter basket, laser)
- Time of first coronary balloon inflation
- Number of pre-dilations or use of direct-stenting
- Type of stent, number of stents, stent diameter and total stent length
- Stent deployment pressure
- Use of post-dilations and maximum pressure on post-dilation
- Use of Intracoronary medications used (e.g. nitroglycerin, nitroprusside, glycoprotein IIb/IIIa inhibitors)
- Pre- and post-TIMI flow grade
- Procedural success (defined as residual stenosis <20% at the end of procedure)
- Time of end of procedure
- Complications
 - Abrupt vessel closure at any time during procedure (main vessel or side branch)
 - Perforation
 - Distal embolization
 - Plaque shift with side branch compromise (>70% residual stenosis in at conclusion of procedure)
 - <TIMI 3 flow in any treated vessel at any time during procedure (also included in post-PCI TIMI flow)
 - Uncovered dissection (grade 3 or higher in vessel >1.5 mm in diameter)
 - Shock during procedure
 - Acute pulmonary edema requiring intubation
 - Dysrhythmia requiring defibrillation

All electronic data is de-identified and resides on password-protected computer. All hard copy of data are secured in a locked cabinet in a locked office.

D. Outcomes

The primary outcome of the overall trial will be peri-procedural myonecrosis using cardiac biomarkers (Troponin I) as defined by the 3rd Universal Definition (42) as follows (biomarkers are evaluated at 6 to 8 hours and 22 to 24 hours post-PCI):

- In subjects with normal baseline cardiac biomarkers, peak post-procedure cardiac biomarker above the 99th percentile upper reference limit
- In subjects with elevated baseline cardiac biomarkers and the biomarker levels are stable or falling, there should be a new cardiac biomarker elevation by $\geq 20\%$ from the most recent pre-procedural level

The primary outcome of the inflammatory marker subset will be change in soluble interleukin-6 level between baseline and 1 hour post-PCI.

Secondary outcomes include:

1) The secondary outcomes of the inflammatory marker substudy will be as follows:

Measurement of other relevant inflammatory markers (such as cell-associated L-selectin (CD62L), cell-associated beta-2 integrin CD11b, soluble L-selectin, myeloperoxidase, elastase, soluble E-selectin, intercellular/vascular cell adhesion molecule-1, white blood cell count, and neutrophil count), as well as mean number of neutrophils adherent to TNF α -stimulated endothelial cells, extent of neutrophil-platelet aggregates, neutrophil extracellular traps (NETs), neutrophil-derived microparticles, tissue factor pathway inhibitor, and endogenous thrombin potential at the end of procedure, 6 to 8 hours post-successful PCI and 22 to 24 hours post-successful PCI.

2) In the overall trial:

a) Occurrence of major adverse cardiac events with composite of the earliest occurrence of death from any cause, non-fatal MI (defined by the 3rd universal definition of MI) (42), or target vessel revascularization (bypass surgery or repeat PCI of the target vessel) assessed at 30 days, 6 months, and yearly for 5 years. Peri-PCI MI will be defined using the Universal Definition (42) (any elevation 5 times the 99th percentile upper limit of normal). No definition currently exists to define post-PCI myocardial necrosis or MI when biomarkers are elevated prior to procedure. In patients with elevated baseline levels of troponin or creatine kinase-MB, peri-procedural MI will be defined as a subsequent increase of more than 2-fold in troponin or creatine kinase-MB from baseline value.

b) Peri-procedural MI will also be defined by the expert consensus document from the Society for Cardiovascular Angiography and Interventions on a new definition of clinically relevant MI after coronary revascularization as follows (43):

- Subjects with normal baseline cardiac biomarkers
 - Peak post-procedure Troponin $\geq 70x$ upper limit of normal or CKMB $\geq 10x$ upper limit of normal
 - A lower threshold (Troponin $\geq 35x$ upper limit of normal, CKMB $\geq 5x$) will be used in subjects with new pathologic Q-waves in ≥ 2 contiguous leads (or new persistent left bundle branch block)
- In subjects with elevated baseline cardiac biomarkers and stable or falling biomarker levels, there should be a new Troponin or CKMB elevation by an absolute increment of $\geq 70x$ (for Troponin) or $\geq 10x$ (for CKMB) upper limit of normal from the most recent pre-procedural level
 - In subjects with elevated baseline cardiac biomarkers and the biomarker levels have not been shown to be stable or falling, there should be a further rise in CKMB (or Troponin) by an absolute increment of $\geq 70x$ for Troponin or $\geq 10x$ (for CKMB) upper limit of normal plus new ST-segment change plus signs consistent with a clinically relevant MI (e.g. new onset or worsening heart failure or sustained hypotension)
- c) The primary outcome of peri-procedural myonecrosis using CKMB as the biomarker

E. Sample Size

A) A total sample size of 400 subjects is needed to demonstrate a 40% reduction in proportion of subjects with peri-procedural myonecrosis (from 30% in the placebo group to 18% in the colchicine group) using a Chi squared test (80% power, 0.05 significance level). This trial is designed to study the effect of colchicine on all-comers to the cath lab. A greater effect is expected on both surrogate and clinical endpoints in the ACS compared with elective PCI population. Our sample size is based on the lowest relative risk reduction observed with anti-inflammatory therapy in elective PCI (19). We approximate that 50% of the patients enrolled will either not have PCI performed after coronary angiography (majority) or will be excluded due to requirement of the use of any of the medications listed in the exclusion criteria prior to post-PCI blood collection (minority). We, therefore, estimate a total sample size of 800 be enrolled in this study to meet the 400 PCI sample size.

B) Sample size for the inflammatory marker subset is calculated based on mean interleukin-6 level of 12pg/mL and standard deviation of 12pg/mL one hour after PCI in predominantly ACS patients reported by Aggarwal et al (3), and an estimated decrease in post-PCI interleukin-6 level by 40% if randomized to colchicine. A 40% decrease is a conservative estimate relative to the 60% reduction of CRP noted in Nidorf's study examining the effect of colchicine in stable CAD patients on aspirin and high-dose statin therapy (38). Furthermore, in Aggarwal's study, interleukin-6 levels increased by 58% from pre-procedural levels (3). Based on the above assumptions, and a two-sided two sample t-test, the number of patients needed in each group to achieve 80% power at the 0.05 significance level is estimated to be 100. A minority of subjects (~16%) may experience no change in IL-6 after PCI (44). To adjust for this possible floor effect, the sample size needed would also be 120 subjects in each group. If a 35% or 30% decrease in post-PCI IL-6 level is observed with colchicine, 129 or 175 subjects will be needed in each group, respectively (80% power, 0.05 significance).

If sample size for the inflammatory marker subset was calculated with surface CD62L (the secondary endpoint) as endpoint, it would be based on the mean (\pm standard deviation) peak post-PCI surface expression of neutrophil CD62L of 9906 ± 6737 MFI (39% rise from pre-PCI) from our pilot AHA-funded project. Based on our volunteer data examining the effects of surface expression of CD62L after a 1.8 mg loading dose and earlier *in vitro* data, we would estimate a decrease in post-PCI CD62L level by ~25% with colchicine. Using a two-sided two sample t-test, the sample size using 80% power and 0.05 significance level would be ~120 subjects in each group. If a 23% or 20% decrease in post-PCI CD62L level is observed with colchicine, 138 or 182 subjects will be needed in each group, respectively (80% power, 0.05 significance).

C) Other secondary endpoints: Sample size for neutrophil-endothelial cell adhesion assay is based on *in vitro* data by our group that demonstrated reductions in neutrophil-endothelial adhesiveness with addition of colchicine (108 ± 11 to 31 ± 5 adherent neutrophils/200x field) (35). Based on a conservative estimate of 20% reduction and a two-sided two sample t-test, the sample size to achieve 95% power (0.05 significance) is ~10 subjects in each group.

Sample size for neutrophil-platelet aggregates is based on data by our group from the peri-procedural glycemic control trial (mean leukocyte platelet aggregation $10.57 \pm 4.10\%$) (45). Based on a ~30% reduction and a two-sided two sample t-test, the sample

size to achieve 95% power (0.05 significance) is ~45 subjects in each group. Since these subjects must meet dual anti-platelet therapy criteria and the probability of one subject satisfying these criteria in the colchicine group is 0.5, increasing the sample size by 10% will make the probability of achieving at least 45 subjects in each group (placebo/colchicine) ~68%. Increasing the sample size to 107 (90*1.10%) will make the probability of achieving at least 45 subjects in each group (placebo/colchicine) ~90%. Subjects in the inflammatory marker substudy that meet dual anti-platelet therapy criteria will undergo neutrophil-platelet aggregate evaluation.

F. Time Period We estimate a weekly enrollment of at least 2 eligible patients who undergo randomization and successful PCI. We believe there will be very low dropout rate due to the excellent side-effect profile and short follow-up which involves blood collection during two time points post-PCI when blood is routinely collected for non-research purposes. Therefore, we believe the study should take approximately 4 years to complete.

G. Follow-up Telephone contact will be made at 30 days, 6 months, and yearly for up to 5 years post-PCI. A subject's participation is voluntary, and the subject has the right to withdraw at any time without penalty or loss of benefit. Should this occur, the reason for the withdrawal will be documented. Discontinuation may be due to subject withdrawal (refuse all subsequent testing/follow-up) or loss to follow-up. Research team will make all reasonable efforts to locate and communicate with subjects who do not complete follow-up but have not officially withdrawn from the study, including the following at each contact time point:

- 1) A minimum of two telephone calls to contact the subject will be attempted
- 2) If these attempts are unsuccessful, a letter will be sent to the subject and the VA electronic medical record system will be reviewed for the needed data
- 3) If the subject misses two consecutive scheduled contact time points, the above-mentioned attempts at communicating with the subject are unsuccessful, and there is no interim data in the VA electronic medical record system the subject will be considered lost to follow-up. As a last resort, the Social Security Death Benefit Index will be queried for death.

2. Data Analysis and Data Monitoring

Categorical variables will be presented as proportions, normally distributed continuous variables as mean \pm standard deviation, and skewed continuous variables as median [interquartile range] (distributions assessed for normality using histogram, quantile-quantile plot and Shapiro-Wilk test). Categorical endpoints will be compared between placebo and colchicine groups using tests of proportions (exact and asymptotic), and continuous endpoints will be compared between placebo and colchicine groups using t-test. Non-parametric alternatives (e.g. Wilcoxon test) and mathematical transformations (e.g. Box-Cox) will be considered as needed for skewed distributions. Statistical significance will be tested using a 2-sided alpha level of 0.05 with appropriate multiple testing correction (Bonferroni or Benjamini-Hochberg) when needed.

To not incur excess bias by deviating from classification according to assigned treatment, an intention-to-treat approach will be utilized as a primary analytic approach. A secondary analysis will be performed utilizing a non-intention-to-treat approach, where subjects are classified according to the treatment actually received and subjects who do

not receive treatment are excluded. We believe crossover and pre-procedure withdrawal will be relatively low. Adopting both approaches, however, will allow for assessment of the robustness of findings.

Pre-specified subgroup analyses will be performed in subjects presenting with versus without ACS and with versus without in-lab complications, as defined below.

In-lab complications: 1) Vessel closure any time during procedure (main vessel or side branch); 2) Perforation; 3) Distal embolization; 4) Plaque shift with side branch compromise (>70% residual stenosis in side branch at conclusion of procedure); 5) <TIMI 3 flow in any treated vessel at any time during procedure (also included in post-PCI TIMI flow); 6) Uncovered dissection (grade 3 or higher in vessel >1.5 mm in diameter); 7) Shock during procedure; 8) Acute pulmonary edema requiring intubation; or 9) Dysrhythmia requiring defibrillation

A) To delineate changes in neutrophil activation during acute vascular injury using a PCI model, markers of neutrophil activity will be assessed by comparison of post- versus pre-PCI samples in the placebo group using paired t-test or paired sample Wilcoxon signed rank test. In addition, to account for all 4 time points (1 pre- and 3 post-PCI), a significant change in markers over time will be determined in the placebo group by estimating speed of change using slope in a linear mixed effects regression model at a 0.05 level of significance cut-off. Markers will then be categorized descriptively as increasing versus not increasing post- compared to pre-PCI.

Univariate and multivariate predictors of markers that demonstrate a significant increase post- compared with pre-PCI (as defined above) will be determined using baseline demographic, clinical, and procedural covariates of interest. Major covariates of interest may include age, sex, race, abdominal circumference, diabetes mellitus, chronic kidney disease, MI within 7 days of PCI, ejection fraction $\leq 40\%$, pre-procedural TIMI 0 or 1 flow, multivessel CAD, visible thrombus or ulcerated lesions, SYNTAX score ≥ 23 , and presence of one of the in lab complications as defined above. Based on our biomedical knowledge and experience in variable selection and statistical modeling, we will balance model richness against a danger to overfit. First, a screening procedure based on the correlation between the outcome and each variable will be used to choose the variables in the multivariate model, and then a stepwise variable selection method including both forward and backward methods will be employed to search for an optimal model gauged by the Akaike information criterion. We will also conduct the principal component analysis for parsimonious modeling, which uses the correlation matrix to identify a small number of components capturing most of the variability and include them in the multivariate logistic regression model.

To determine changes in subsequent cellular interactions, neutrophil adhesion to endothelial cells and neutrophil-platelet aggregates will be assessed post- compared with pre-PCI samples in the placebo group using paired t-test or paired sample Wilcoxon signed rank test.

Finally, a mediation analysis will be performed in a logistic regression model to test the hypothesis that an increase in markers of neutrophil activation is associated with peri-procedural myonecrosis, and that this association is mediated by neutrophil-endothelial cell and neutrophil-platelet adhesion.

Similar analyses will be performed in the evaluation of the change in NETs and neutrophil-derived microparticles post- versus pre-PCI. A mediation analysis will be performed in a logistic regression model to test the hypothesis that an increase in these neutrophil extracellular fragments is associated with peri-procedural myonecrosis, and that this association is mediated by tissue factor pathway inhibitor/thrombin generation.

B) To determine the effect of colchicine on changes in markers of neutrophil activation and subsequent cellular interactions during acute vascular injury, pre- to post-PCI changes will be examined between the placebo and colchicine groups using two-sample t test or Wilcoxon rank sum test. To further study the time-varying pattern of treatment effect, with the observations at pre-PCI and multiple post-PCI time points, a repeated measurements or longitudinal data analysis modeling will be used by incorporating both individual-specific terms and the treatment by time interaction. The Analysis of Variance Analysis will be conducted using the Generalized Estimating Equations or Quasi-Likelihood approach. Alternatively, proportion of subjects with significantly increased markers of neutrophil activity post- compared to pre-PCI (defined above using estimated slope of change) will be compared between the placebo and colchicine groups using tests of proportions. Finally, an interaction with pre-procedural use of colchicine versus placebo will also be evaluated to determine whether or not colchicine modulates the mediation analyses described above.

C) To explore the association between markers of neutrophil activation and outcomes after acute vascular injury, the association between tertiles of markers of neutrophil activity at baseline and dichotomous outcome of peri-procedural myonecrosis will be assessed using logistic regression modeling and presented as odds ratio and 95% confidence interval. Similar analyses will be performed to evaluate the association between tertiles of markers of neutrophil activity at baseline and dichotomous outcome of peri-procedural MI, as well as long-term composite of all-cause mortality, non-fatal MI, and target vessel revascularization.

However, the capacity for neutrophil activity to increase in the setting of acute vascular injury may be more pertinent than baseline activity. Therefore, using the estimated speed of change in neutrophil activity over time from pre- to post-PCI, markers of neutrophil activity will be categorized as increasing or non-increasing (no change or decreasing) based on analysis of slope and a 0.05 level of significance cut-off. The association between increasing versus non-increasing markers of neutrophil activity and peri-procedural myonecrosis will be evaluated using logistic regression modeling (increasing marker as main predictor, peri-procedural myonecrosis as dichotomous outcome). Similar analyses will be performed to evaluate the association between increasing versus non-increasing markers of neutrophil activity and peri-procedural MI, and long-term composite of all-cause mortality, non-fatal MI, and target vessel revascularization.

Logistic regression models will be built hierarchically to include main covariates. As above, age, sex, race, abdominal circumference, diabetes mellitus, chronic kidney disease, MI within 7 days of PCI, ejection fraction $\leq 40\%$, pre-procedural TIMI 0 or 1 flow, multivessel CAD, visible thrombus or ulcerated lesions, SYNTAX score ≥ 23 , and presence of one of the in lab complications as defined above. Based on our biomedical knowledge and experience in variable selection and statistical modeling we will balance model richness against a danger to overfit as described above.

Finally, to explore the effects of colchicine on peri-procedural myonecrosis in a PCI model, the rate of peri-procedural myonecrosis and peri-procedural MI in subjects randomized to the placebo vs colchicine groups will be assessed using the tests of proportions. This association will also be assessed using logistic regression modeling and will include the main covariates described above.

D) The low-dose colchicine regimen in the current proposal has been studied and demonstrates an excellent side effect profile. A Data Safety Monitoring Committee (DSMC) will be formed to review ongoing safety data. This committee will be a group consisting of three physicians and, when needed, one biostatistician. The DSMC meetings will include an initial open meeting for discussion with the Principal Investigator and the presentation of reports by the study coordinator followed by a closed meeting of voting DSMC members. The DSMC will review blinded safety data three times a year (or more often as necessary) to ensure the safe and proper treatment of subjects. The committee will also review recruitment data, study subject withdrawals, data on drug tolerability, and protocol violation data to determine whether any substantial deviations from the initial study plan might alter the original risk benefits analysis. If the frequency and/or severity of adverse events are thought to be study related, or if other logistical issues related to study drug tolerability, are thought to compromise the integrity of the study protocol, the DSMC can make recommendations for protocol modification or early termination of the study. After each meeting, the committee will prepare a brief report to be submitted to the Principal Investigator that will recommend: 1) study continuation without modification, 2) study continuation with modification, or 3) study discontinuation. A recommendation of study modification or study discontinuation will lead to an immediate cessation of study enrollment, but in the event of a recommendation of study modification, study enrollment may be allowed to resume once the human subjects safety issues and/or other logistical issues have been adequately addressed to the satisfaction of the DSMC and IRB. In the event of identification of any relevant safety findings that may change the original risk benefit analysis for the human subjects in the study, these findings will be forwarded to the IRB committee and will be incorporated into a revised protocol and consent form. All current participants and future participants will be required to sign the revised consent form. The Principal Investigator will be responsible for reporting of adverse events to the DSMC and VA IRB according to standard definitions.

3. Data Storage and Confidentiality

All patient data will be kept strictly confidential, except when published for purposes of reporting data. In that case, the patients are never identified. As noted previously, all electronic data will be de-identified and resides password-protected computers. All hard copy of data will be secured in a locked cabinet in a locked office on Manhattan VA Hospital property. All biological specimens will be encoded with no identifying information and stored at NYU School of Medicine Smilow Research Building that can only be traced back to the individual by the research team. There is a minimal risk of loss of confidentiality though every precaution will be taken to avoid this. The statistician will have access to only the de-identified data for analysis.

IV. Risk/Benefit Assessment

1. Risk

The proposed colchicine regimen for this proposal has been studied in regards to safety profile, and the adverse event profile was similar to that of placebo, with 23% of patients having diarrhea and none having severe diarrhea or vomiting (36). There is also a rare incidence of hypersensitivity to colchicine that may cause skin rash, hives, and/or angioedema.

Venipuncture is associated with risk of pain, bruising, infection, and less commonly development of anemia, lightheadedness or syncope. We will be collecting blood at 4 well-spaced time points.

2. Protection against risks

The study investigators are specialists in Interventional Cardiology with particular training in radiation safety. Subjects will have access to study investigators at all times by telephone.

3. Potential Benefits to the Subjects

Subjects may experience no direct benefit.

V. Investigators Qualifications and Experience

Attached are copies of the curriculum vitae for each study investigator. The investigators each have completed training in the protection of human subjects.

VI. Subject Identification, Recruitment, and Consent

1. Method of Subject Identification and Recruitment

Subjects will be screened and recruited when they are referred to the Manhattan Veterans Affairs Hospital, Bellevue Hospital Center, and NYU Langone Medical Center cardiac catheterization laboratory for clinically indicated coronary angiography. Informed consent will be obtained by the study team at least 2 hours prior to coronary angiography. Every effort will be made to recruit subjects prior to the day of the catheterization whenever practical.

2. Process of Consent

Subjects will be provided the required information for informed consent in writing, and they will also be given an oral description of the project. Consent will be obtained by the study investigators who have training in obtaining consent from research subjects.

Completed written consent forms will be stored securely with the other study documents by the Principal Investigator.

3. Subject Capacity and Comprehension

Only subjects with the capacity to give consent will be included in the study. The subjects will be required to state their understanding of each step of the informed consent and to demonstrate their understanding by repeating the information provided.

4. Consent forms

A study consent form is attached.

5. Costs to the Subject

The subjects will incur no costs as a result of study participation.

6. Payment for Participation

The subjects will receive no payment of any sort for participating in the study.

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