**Appendix Table E49. Phenotypic test details in studies assessing the predictive ability of VASP in patients with ischemic heart disease**

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| --- | --- | --- | --- | --- | --- | --- |
| **Author, year [ref]**  **UID**  **Country**  **Study Name** | **Test/Device name**  **Device category Device name & manufacturer\*** | **Agonist used** | **Sample Collection and Procurement**  **Anticoagulant used**  **Interval between clopidogrel doses and blood sampling (in days)**  **Interval between sampling and testing (in days):** | **Grouping of Phenotypes\*\* [Definition]** | **Rational for the grouping of phenotypes reported (Yes/No)**  **[short description]** | **Frequency of phenotypes** |
| Freynhofer, 2011{Freynhofer, 2011 1 /id}  21614416  Austria  NR | Flow cytometric analysis of VASP phosphorylation  Platelet VASP kit  Biocytex, Marseille, France | ADP | Venous blood samples along with the routine blood samples were collected via atraumatic venipuncture of the forearm into coagulation tubes  buffered sodium citrate 3.2%  6-24 h (morning) after PCI  48 hours after blood collection | VASP result: PRI≤60.2% (low reactivity, good response)  VASP result: PRI>60.2% (high reactivity, poor response) | ROC curve (area under the ROC-curve was 0.683 (p=0.014) for the platelet reactivity index (PRI) calculated from median fluorescence intensities (FI) with an optimal cut-off at 60.2% PRI.) | VASP result: PRI≤60.2% (low reactivity, good response): 114/300 (38%)  VASP result: PRI>60.2% (high reactivity, poor response): 186/300 (62%) |
| Siller-Matula,  2009{Siller-Matula, 2009 234 /id}  19135705  Austria  NR | Flow cytometric analysis of VASP phosphorylation  Platelet VASP kit  Biocytex, Marseille, France | Adenosine diphosphate  (ADP), prostaglandin (PGE1) | 1st blood sample: in catheterization laboratory, after PCI and after 250 mg IV aspirin ; 2nd blood sample: 20‑24 hours after PCI  3.8% citrate  NR; Clopidogrel came first  0.04 days (1 hour) | Platelet reactivity index ≥ 69% by VASP (? Low efficiency)  Platelet reactivity index <69% by VASP | Based on normal ranges as reported by manufacturer | Platelet reactivity index ≥ 69% by VASP (? Low efficiency): 8 (27%)  Platelet reactivity index <69% by VASP: 22 (63%) |
| Blindt, 2007{Blindt, 2007 189 /id}  18064332  Germany  NR | Flow cytometric analysis of VASP phosphorylation  NR  Biocytex, Marseille, France | 10 µM ADP | blood samples were obtained 72–96 h after stent placement  Citrate  Interval at least 8 days.  NR | VASP-platelet reactivity indices (VASP-PRI) > 48%  VASP-platelet reactivity indices (VASP-PRI) < 48% | ROC curve revealed VASP PRI <48% be the best cut-off | VASP-platelet reactivity indices (VASP-PRI) > 48%: NR  VASP-platelet reactivity indices (VASP-PRI) < 48%: NR |
| Kalantzi, 2011{Kalantzi, 2011 19 /id}  21255245  Greece  NR | Flow cytometric analysis of VASP phosphorylation  NR  Biocytex, Marseille, France | ADP | Pre-clopidogrel, 5 days after clopidogrel, 30 days after clopidogrel  Citrate  5 days; the pre-clopidogrel sampling was not used for estimation of responder status  NR | Non-responders (VASP platelet reactivity index [PRI] ≥50%)  Responders (VASP PRI < 50%) | Based on literature; Proposed previously [Ref #6] and also associated with adverse clinical outcome [ref #4] | Non-responders (VASP platelet reactivity index [PRI] ≥50%): 46 (75.4%)  Responders (VASP PRI < 50%): 15 (24.6%) |
| Siller-Matula, 2010{Siller-Matula, 2010 89 /id}  19943879  Austria  NR | Flow cytometric analysis of VASP phosphorylation  NR  Biocytex, Marseille, France | ADP | Blood samples obtained from arterial sheath  3.8% citrate  Dose first (clopidogrel loading dose given at least 2 hr before PCI); Samples taken directly after PCI and at least 5 min after IV aspirin dose (250 mg).  NR | Clopidogrel responders (PRI<42%)  Clopidogrel nonresponders (PRI≥42%) | ROC optimal cutoffs for prediction of definite stent thrombosis at 6 m, as done in this study | Clopidogrel responders (PRI<42%): 154 (37%)  Clopidogrel nonresponders (PRI≥42%): 262 (64%) |
| Bjelland, 2010{Bjelland, 2010 42 /id}  20727659  Norway  NR | Dual flow cytometry  PLT VASP/P2Y12  Biocytex, France | ADP | Whole blood; Day 1 and Day 3 after clopidogrel administration  citrate  Day 1: Median 21 hrs [IQR: 17.5-27.5]  Day 1: Median 68 hrs [IQR: 63-72]  NR | Satisfactory clopidogrel effect (Platelet reactivity index <0.5)  Unsatisfactory clopidogrel effect (Platelet reactivity index ≥0.5) | Based on literature | Satisfactory clopidogrel effect (Platelet reactivity index <0.5): 0 (0%)  Unsatisfactory clopidogrel effect (Platelet reactivity index ≥0.5): 25 (100%) |
| Bonello, 2007{Bonello, 2007 199 /id}  17488353  France  NR | VASP  Platelet VASP kits  Diagnostica Stago,Asnieres, France | ADP and/or prostaglandin E1 (PGE1) | Blood samples for PRI testing were drawn before PCI and at least 24 h after clopidogrel loading dose and aspirin  3.8% trisodium citrate  24 hours  24 hours | Quintile 1 (<47%) vs Quintile 2-4 | 70% is normal range using ROC curve. | Quintile 1 (<47%) : 29  vs Quintile 2-4: 115 |
| Djukanovic, 2008{Djukanovic, 2008 163 /id}  18719318  Serbia  NR | Flow cytometric analysis of VASP phosphorylation  Platelet VASP kit  Diagnostica Stago (Biocytex), Asnières, France | PGE1 and ADP | Whole blood; Collection time NR  0.129 M sodium citrate  NR  NR | At Day 2:  Rapid response (Platelet reactivity index ≤50% at 2 days)  Not rapid response (Platelet reactivity index >50% at 2 days)    At Day 7:  Good responders (Platelet reactivity index ≤50% at Day 7): NR (Difficult to extract from Fig 2B)  Bad responders (Platelet reactivity index >50% at Day 7) | Based on literature | Rapid response (Platelet reactivity index ≤50% at 2 days): 7/17 (41.2%)  Not rapid response (Platelet reactivity index >50% at 2 days): 10/17 (58.8%)    Good responders (Platelet reactivity index ≤50% at Day 7): NR (Difficult to extract from Fig 2B)  Bad responders (Platelet reactivity index >50% at Day 7): NR (Difficult to extract from Fig 2B) |
| El Ghannudi, 2011{El, 2011 3 /id}  21524751  France  NR | Flow cytometric analysis of VASP phosphorylation  Platelet VASP kit  Diagnostica Stago (Biocytex), Asnières, France | ADP | Whole blood samples were drawn by venous puncture  0.129 M sodium citrate  Blood taken at least 6 h after clopidogrel, mean interval ~24 h, and range 12-120 h  Sent to Alsace laboratory for VASP immediately after sample obtained but interval NR | Non-diabetic responders (NDM-R) (PRI <61%)  Non-diabetic low responders (NDM-LR) (PRI≥61%)  Diabetic responders (DM-R) (PRI<61%)  Diabetic low responders (DM-LR) (PRI≥61%)  [NB outcome data available for only 429 of the 436 (98.4%) but NR why]  Also some data reported for groups with cutoff at 60%, not 61%, but no explanation given | Based on literature (Ref 7) | Non-diabetic responders (NDM-R) (PRI <61%) 177 (64.8%)  Non-diabetic low responders (NDM-LR) (PRI≥61%) 96 (35.2%)  Diabetic responders (DM-R) (PRI<61%) 85 (52.1%)  Diabetic low responders (DM-LR) (PRI≥61%) 78 (47.9%) |
| El Ghannudi, 2010{El, 2010 74 /id}  20630458  France  NR | Flow cytometric analysis of VASP phosphorylation  Platelet VASP kit  Diagnostica Stago (Biocytex), Asnières, France | ADP | Blood samples drawn at least 6 h after a loading dose (300 or 600 mg) of clopidogrel.  0.129 mol/L sodium Citrate  6 h after a loading dose (300 or 600 mg) of clopidogrel.  NR | Low responder (PRI≥61%)  Responder (PRI≥61%) | Based on an ROC curve from data on subjects in this study | Low responder (PRI≥61%): N=184  Responder (PRI≥61%): N=277 |
| Morel, 2011{Morel, 2011 187 /id}  21251579  France  NR | Flow cytometric analysis of VASP phosphorylation  Platelet VASP kit  Diagnostica Stago (Biocytex), Asnières, France | prostaglandin E1 (PGE1) or ADP | after PCI  0.129 M sodium citrate  0.25 (6 hours after loading dose)  NR [It is reported that the samples were sent off immediately for analysis] | Low responders (PRI≥ 61%)  Normal responders (PRI<61%)  By Quartile:  Quartile 1 (<40.30%)  Quartile 2 (40.30%–55.83%)  Quartile 3 (55.84%–70.25%)  Quartile 4 (>70.25%) | Single PRI cutoff based on literature; quartile cutoffs not explicitly reported | Low responders (PRI≥ 61%): 173 (40%)  Normal responders (PRI<61%): 260 (60%)  Quartile 1 (<40.30%): 108 (25%)  Quartile 2 (40.30%–55.83%): 108 (25%)  Quartile 3 (55.84%–70.25%):108 (25%)  Quartile 4 (>70.25%): 109 (25%) |
| Palmerini, 2010{Palmerini, 2010 81 /id}  19604542  Italy  DOUBLE | VASP Phosprylation  VASP kit  Biocytex, Marseille, France | ADP 20 µmol/L | 1-2 hrs after ingestion of last clopidogrel dose done at baseline, 1 week and 1 month  Citrate  NR [clopidogrel]  0.02 [30 minutes] | Poor responders (PRI>50%) in clopidogrel 75 mg group  Normal responders (PRI between 30-50%) in clopidogrel 75 mg group  Poor responders (PRI>50%) in clopidogrel 150 mg group  Normal responders (PRI between 30-50%) in clopidogrel 150 mg group | Based on literature | Poor responders (PRI>50%) in clopidogrel 75 mg group: 21 (87.5%)  Normal responders (PRI between 30-50%) in clopidogrel 75 mg group : 3 (12.5%)  Poor responders (PRI>50%) in clopidogrel 150 mg group: 20 (83.3%)  Normal responders (PRI between 30-50%) in clopidogrel 150 mg group: 4 (16.7%) |
| Schafer, 2011{Schafer, 2011 11 /id}  21655677  Germany  NR | Flow cytometric analysis of VASP phosphorylation  Platelet VASP/P2Y12 kit  Biocytex, Marseille, France | ADP | NR  NR  24 hours after loading with 600 mg clopidogrel  NR | PRI >50% (suboptimal inhibition; impaired clopidogrel responsiveness)  PRI≤ 50% 14 (26%)  PRI quartiles: <51%, 51-58%, 59-71%, and >71%  PRI >57%  PRI ≤57% | >50% vs. </=50% was based on literature  57% threshold based on ROC analysis in present study | PRI >50% (suboptimal inhibition; impaired clopidogrel responsiveness): 40 (74%)  PRI≤50%: 14 (26%)  PRI quartiles: <51%, 51-58%, 59-71%, and >71%: Frequency NR  PRI >57%: 40 (74%)  PRI ≤57%: 14 (26%) |
| Frere, 2007{Frere, 2007 193 /id}  17938809  France  NR | Flow cytometric analysis of VASP phosphorylation  Platelet VASP/P2Y12 kit  Biocytex, Marseille, France | PGE1 and ADP | before the PCI at least 12 h after the loading dose of clopidogrel and aspirin, and before administration of tirofiban if needed.  3.8% trisodium citrate  After 12hours after the loading dose of clopidogrel  With 1 hour | VASP PRI ≥ 53%  VASP PRI < 53% | As per the ROC curve created using data from study subjects | VASP PRI ≥ 53%: 13/106 (12.3%)  VASP PRI < 53%: 1/89 (1.1%) |
| Kalantzi, 2012{Kalantzi, 2012 18174 /id} 21806493  Greece  NR | VASP  VASP/P2Y12 kit (BioCytex, Marseille,  France) | ADP 2.5, 5 and 10uM  ADP  ADP | Citrated blood samples were collected after the patient’s presentation at the emergency room  before clopidogrel administration (baseline), as well as at 5- and 30-days after clopidogrel loading.  citrate  5 days  30 days | nonresponder  VASP PRI >50%  responder  VASP PRI <50% | reference 15, 23 | nonresponder n=12  responder n=28 |
| Siller-matula, 2012{Siller-Matula, 2012 18177 /id}  22260716  Austria  PEGASUS-PCI | VASP  Platelet VASP; BioCytex,  Marseille, France | ADP | Blood samples from patients  were obtained from the arterial sheath (6F) in the catheterization  laboratory directly post-PCI and at least 5 min after  intravenous infusion of aspirin.  3.8% sodium citrate  NR  performed up to 24 h after blood sampling | Clopidogrel  non-responder  according to MEA (≥ 48 U)  Clopidogrel responder  according to MEA  (< 48 U)  n = 321 (80%) | ref 16, 28 | non-responder  n = 81 (20%)  responder  n = 321 (80%) |
| Tselepis, 2011 {Tselepis, 2011 1 /id}  22008470  Greece  NR | VASP  FACS Calibur flow cytometer  Becton Dickinson, san Jose, CA, USA  VASP/P2Y12 kit (BioCytex, Marseille,France) | ADP+PGE1 (prostaglandin E1) | Blood samples were obtained from all patients before clopidogrel loading (baseline) as well as at 5 and 30 days afterwards.  NR  0, 5 and 30 days  3h after blood sampling | clopidogrel responders  PRI 50% as cutoff | reference 21 | clopidogrel responder n=57  non-responder n=17 |
| Gaglia, 2012{Gaglia, 2011 18244 /id}  21919956  USA  NR | VASP  FACS Calibur flow cytometer  Becton Dickinson, san Jose, CA, USA  VASP/P2Y12 kit (BioCytex, Marseille,France) | ADP+PGE1 (prostaglandin E1) | 6 hours following a loading  dose of clopidogrel  3.2% sodium citrate  6 hours  6 and 24 hours following PCI | PRI>50%  PRI≤50% | Based on literature | PRI>50%: 79  PRI≤50%: 121 |
| Cuisset, 2011{Cuisset, 2011 18245 /id}  21872198  France  NR | VASP  Platelet VASP; BioCytex,  Marseille, France | ADP | ≥12 hours after the loading dose of aspirin and clopidogrel  3.8% trisodium citrate  12 hrs  NR | PRI>50%  PRI≤50% | NR | PRI>50%: 331  PRI≤50%: 358 |

\*If more than one test, use separate rows

\*\*E.g., nonresponsive vs. responsive to clopidogrel, high vs. low platelet reactivity,

ADP= adenosine 5'-diphosphate; Ag= aggregation; PGE1=prostaglandin; ROC=receiver operating characteristic; AUC=area under the curve; IPA= inhibition of platelet aggregation; LTA= light transmission aggregometry; MEA= multiple electrode platelet aggregometry; PFA= platelet function analysis; TEG=thromboelastography; sTEG=short thromboelastography; VASP = vasodilator-stimulated phosphoprotein; VASP-FCT=vasodilator-stimulated phosphoprotein flow cytometry; CEPI=collagen-epinephrine ; CADP=collagen-ADP; CT=closure times; HCPR=high on-clopidogrel platelet reactivity; PCI = percutaneous coronary intervention; RPA= residual platelet aggregation; GP= glycoprotein; HRP=high platelet reactivity; NPR=normal on-treatment platelet reactivity; HPPR= high post-treatment platelet reactivity; MPA= maximum platelet aggregation; RPR= residual platelet reactivity; OTPR=on-treatment platelet reactivity; DPAI= degree of platelet aggregation inhibition; PRU=P2Y12 reaction units; CRP=C-reaction protein; PRI=platelet reactivity index; LR=low responder; IQR=interquartile range; AA= arachidonic acid; LD=loading dose; MD=maintain dose; SD=standard deviation; NR=not reported;