HEALTH SERVICES LABORATORIES

# Assessment of CYP2C19 genotype testing on point of care platform and laboratory based genetics with supplementary platelet function studies

Sabina McCann[1], Arvind Chandratheva[2], Deepak Singh[1]

1. Haemostasis – Health Service Laboratories, London WC1H 9AX **2.** Institute of Neurology Stroke Research Centre UCL, University College London, London, UK.

## Introduction

In the United Kingdom there are over 100,000 hospital admission for strokes per year[1].To reduce the risk of secondary stroke the National Institute for Health and Care Excellence (NICE) recommend the use of 75mg clopidogrel daily. NICE guidelines (2024) for the use of clopidogrel recommend CYP2C19 genotype testing prior to administration of the drug[2].

To date, it is believed that the effectiveness of clopidogrel is dependent on an individual's CYP2C19 gene status, however, we demonstrate that the administration and/or cessation of clopidogrel should not be based solely on the CYP2C19 genotype result without parallel platelet function testing to confirm P2Y12 receptor status.

## Background

Clopidogrel is an antiplatelet drug that blocks the P2Y12 receptors on the surface of platelets thus preventing adenosine diphosphate (ADP) from binding inhibiting platelet activation and aggregation. Clopidogrel is converted to its active form via cytochrome CYP450 enzymes (2C19) in the body.

Alleles of the CYP2C19 gene are categorised into functional groups due to the predicted phenotype. Patients with a loss of function (LOF) of one allele have a decreased enzyme activity, and those with LOF of two alleles have a dramatically reduced enzyme activity resulting in poor efficacy of clopidogrel and therefore are at an increased risk of further thrombotic events.

## Investigation

- Subjects identified by the UCLH Stroke team as high risk due to reoccurring transient ischemic attack (TIA). TIA event not within 14 days of testing.
- CYP2C19 genotype testing comparison on two platforms; Genedrive® point of care testing (POCT) and laboratory-based genetics. Genotype results and subsequent phenotype from both platforms expected to be identical.
- Comparison of CYP2C19 phenotype with existing standard of care (SOC) platelet function tests (PFT) as the inhibition of the P2Y12 receptor for patients on clopidogrel is confirmed by PFT under shear stress using the PFA P2Y test. Expected results detailed in table 3.
- Confirmation of PFA P2Y result due to test limitations (high platelet count >450x10$^9$/L, high haematocrit (>0.400 L/L) and/or raised von Willebrand antigen >1.60 IU/ml) via platelet aggregation studies.
- All pre-analytical variables considered and excluded prior to processing all samples. Sodium citrate samples collected after 2 to 5 hours post dose, and tested within 4 hours of sample collection.

## Materials & Methods

### *Platelet Function Assay (PFA)*

Siemens Innovance® PFA-200 system. Citrated blood was subjected to high shear at 37°C within a capillary tube with Innovance® PFA P2Y test cartridges. The membrane of INNOVANCE® PFA P2Y is coated with 20 µg ADP, 5 ng prostaglandin and 459 µg calcium chloride. The aperture closure time (CT) recorded in seconds.

### *Platelet aggregation*

Platelet aggregometry analysed using a PAP8E profiler. The following platelet agonists were employed at specific concentrations: arachidonic acid (AA) 1.0 Mm, ADP 5.0µM and 10µM. The maximum aggregation (MA) and final aggregation (FA) are recorded in percentage after 6 minutes of aggregation at 37°C.

### *Lab based genetics*

xTAG CYP2C19 kit (volume 3) multiplexed nucleic acid test. Detection of *1, *2, *3, *4, *5, *6, *7, *8, *9, *10 and *17 alleles on Luminex® 200™ platform. EDTA samples.

### *Genedrive® POCT*

Genedrive® CYP2C19 ID kit used in conjunction with the automated Genedrive® system for the detection *2, *3, *4, *8, *17 and *35 alleles of the CYP2C19 gene in human buccal cells.

## Results

37 patient samples were processed for CYP2C19 genotype testing and SOC platelet function assessment. Patient demographics are detailed in Table 1. Additional anti-platelet drugs were documented as well as administration of proton pump inhibitors (PPI) that are often prescribed alongside clopidogrel to reduce the adverse affects of the drug[3] .

The CYP2C19 genotype analysis on both platforms are detailed in table 2, highlighting the identical results for 35 of the 36 patients tested. For one patient the Genedrive® POCT resulted a *3/*35 genotype concluding a poor metaboliser, however, the lab-based genetics resulted a *1/*3 genotype and therefore an intermediate metaboliser.

One patient did not have lab-based genetic CYP2C19 testing.

### *Patient Summary*

| Sex | | | | | | | |
|---|---|---|---|---|---|---|---|
| Male | | | | Female | | | |
| 26 | | | | 11 | | | |
| **Age (Years)** | | | | | | | |
| 20 -29 | 30 - 39 | 40 -49 | 50 - 59 | 60 -69 | 70 -79 | 80 -89 | 90- 99 |
| 1 | 2 | 5 | 6 | 10 | 8 | 4 | 1 |
| **Ethnicity** | | | | | | | |
| White - British | White - European | White - Other | Black - African | Black - Caribbean | Asian | Not Disclosed | |
| 19 | 2 | 4 | 3 | 1 | 5 | 3 | |
| **PPI Status** | | | | | | | |
| On PPI | | | No PPI | | Not disclosed | | |
| 21 | | | 8 | | 8 | | |
| **Aspirin Status** | | | | | | | |
| On Aspirin | | | No Aspirin | | | | |
| 20 | | | 17 | | | | |

**Table 1.** Summary of patient demographic data collected (n =37).

### *Comparison of CYP2C19 Genetic Results*

| CYP2C19 Phenotype | CYP2C19 Genotype | Number of Results | |
|---|---|---|---|
| | | Genedrive® POCT | Lab-based Genetics |
| Ultra rapid | *17/*17 | 2 | 2 |
| Rapid | *1/*17 | 7 | 7 |
| Normal | *1/*1 | 13 | 13 |
| Intermediate | *1/*2 | 10 | 10 |
| | *1/*3 | 0 | 1 |
| | *2/*17 | 1 | 1 |
| Poor | *2/*2 | 2 | 2 |
| | *3/*35 | 1 | 0 |

**Table 2.** Comparison of genotype results between Genedrive® POCT and lab-based genetics.

### *CYP2C19 Phenotype vs Platelet Function*

Summary of the PFA P2Y results are displayed in figure 1 with conflicting CYP2C19 pehnotype and PFA P2Y results highlighted. The P2Y results for 5 intermediate phenotype (*1/*2, *2/*17) are >300 seconds indicating complete inhibition of the P2Y12 receptor. Additionally, for 2 normal metaboliser (*1/*1) patients there is no inhibition of the P2Y12 receptor demonstrated through the P2Y results of <100 seconds. Expected PFA P2Y results and P2Y12 inhibiton are detailed in table 3.

All unexpected PFA P2Y results and P2Y12 receptor status' were confirmed via platelet aggregation studies.

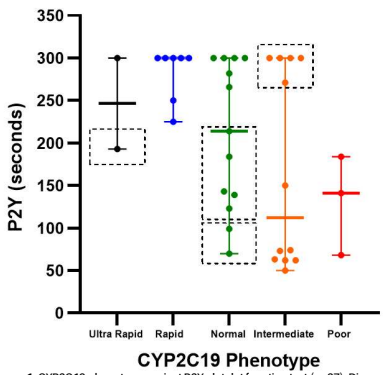


**Figure 1.** CYP2C19 phenotype against P2Y platelet function test (n=37). Discordant results highlighted.

| CYP2C19 Phenotype | Expected P2Y Result (seconds) | P2Y12 Receptor | Number of Discordant Results |
|---|---|---|---|
| Ultra rapid | >300 | Complete Inhibition | 1 |
| Rapid | >300 | Complete Inhibition | 0 |
| Normal | >300 | Complete Inhibition | 6 |
| Intermediate | 107-199 | Mild Inhibition | 5 |
| Poor | <106 | No Inhibition | 0 |

**Table 3.** Expected P2Y results per CYP2C19 phenotype.

## Discussion

The Genedrive® POCT and lab-based genetics for CYP2C19 produced identical results for 35 of 36 patients tested. The discrepant result can be attributed to the limitation of the xTAG CYP2C19 kit not being able to detect *35 allele. Although *35 allele is rare, the LOF of *35 allele has a reduction in enzyme activity and thus the efficacy of clopidogrel. In this case the metaboliser status of the patient was confirmed via PFTs through the PFA P2Y result of 68 seconds.

Assessment of P2Y12 receptor via platelet aggregation studies confirmed the PFA-P2Y results, but, when subject is also on aspirin it is difficult to differentiate if reduced maximum aggregation and subsequent disaggregation is due to the clopidogrel or aspirin. In such cases, discussion with clinical team is required to re-arrange testing.

Current NHS and NICE guidelines state that CYP2C19 normal metabolisers should receive standard dose clopidogrel and CYP2C19 intermediate metabolisers should avoid clopidogrel administration if possible. However, we have presented several cases whereby the administration or cessation of clopidogrel cannot be determined by CYP2C19 genetic analysis alone. Proposed testing algorithm detailed in figure 2.

Further investigation is required to evaluate the use of PPIs, specifically lansoprazole alongside clopidogrel with regards to each CYP2C19 genotype.

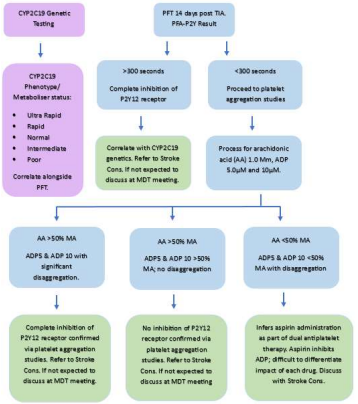


**Figure 2.** Proposed clopidogrel resistance algorithm.

## Conclusion

- The Genedrive® POCT and lab-based genetics for analysis of CYP2C19 genotype are in agreement with a 97% correlation.
- CYP2C19 genotype and phenotype testing requires additional platelet function testing to confirm P2Y12 receptor inhibition.
- Proposed testing algorithm for confirmation of clopidogrel resistance detailed in figure 2.

## References

**1.** King D, Wittenberg R, Patel A, *et al*: The future incidence, prevalence and costs of stroke in the UK. *Age Ageing*. 2020; **49**(2): 277–282

**2.** National Institute for Health and Care Excellence (2024) CYP2C19 genotype testing to guide clopidogrel use after ischaemic stroke or transient ischaemic attack [NICE Guideline No. DG59]. https://www.nice.org.uk/guidance/dg59

**3.** Pang, J., Wu, Q., Zhang, Z., Zheng, T.Z., Xiang, Q., Zhang, P., Liu, X., Zhang, C., Tan, H., Huang, J. and Liu, W., 2019. Efficacy and safety of clopidogrel only vs. clopidogrel added proton pump inhibitors in the treatment of patients with coronary heart disease after percutaneous coronary intervention: a systematic review and meta-analysis. *IJC Heart & Vasculature*, *23*, p.100317.