HEALTH SERVICES LABORATORIES

The role of CYP2C19 genetic testing in Clopidogrel therapy

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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) also recommend CYP2C19 genotype testing[2]. 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. Despite compliance with treatment, reoccurrence of stroke is occurring in some patients and functional platelet testing is employed to assess the possibility of clopidogrel resistance.

We aim to study whether the administration and/or cessation of clopidogrel should be based solely on the CYP2C19 genotype and predicted phenotype result without supplementary platelet function testing to confirm P2Y12 receptor status.

Background

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. The 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.

The aim of the study is to confirm predicted metaboliser status derived from CYP2C19 genetic results through functional phenotype testing.

Investigation

- Subjects identified by the UCLH Stroke team as high risk based on 1. recurrent transient ischemic attack (TIA), 2. large artery stenosis at high risk of reoccurrence, 3. recent stent and 4. ethnicity.
- CYP2C19 genotype testing comparison on two platforms using Rapid point of care device (POCT) and laboratory-based genetics.
- Comparison of CYP2C19 genetic phenotype with functional CYP2C19 resistance using the PFA P2Y test, a shear stress P2Y12 receptor assessment.
- 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 using Innovance® PFA P2Y test cartridges(20 µg ADP, 5 ng prostaglandin and 459 µg calcium chloride) The aperture closure time (CT) recorded in seconds. Local result interpretation guide below:

PSY Results (secs)	P2Y12 Receptor	Clopidogrel Resistance	Follow Up Protocol
> 300	Complete Inhibition	No	Nil
201 - 299	Moderate Inhibition	Unlikely	Exclude PPI interference and/ or raised VWF, timing of bloods, drug dosage checks. Confirm with repeat P2Y and platelet aggregation tests.
107 - 200	Mild Inhibition	Possible	
< 106	No Inhibition	No	

Platelet function confirmation assays

Platelet aggregometry analysed on the PAP8E profiler using platelet agonists of the ADP receptor where maximum aggregation (MA) and disaggregation was used to determine the extent of secondary phase inhibition, agonist to arachidonic acid (AA) used to determine concurrent aspirin intake. vWF antigen using Sysmex CS2500 analyzers, and platelet counts and platelet clumping checks using Sysmex XN-20 analysers and manual microscopy respectively.

Lab based genetics

xTAG CYP2C19 kit (version 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. Processed by the HSL Dept. of Genetics and Molecular Pathology.

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.

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

Asprin Status	
On Asprin	**No Asprin**
20	17

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

Comparison of CYP2C19 Genetic Results

Predicted CYP2C19 Phenotype	CYP2C19 Genotype	NUMBER OF RESULTS	
		Genedrive® POCT	Lab-Based Genetics
Ultra Rapid	⁎7 / ⁎17	2	2
Rapid	⁎1 / ⁎17	7	7
Normal	⁎1 / ⁎1	13	13
Intermediate	⁎1 / ⁎2	11	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 Predicted Phenotype vs Platelet Function

Summary of the PFA P2Y results are displayed in Figure 1 showing conflicting CYP2C19 predicted phenotype and PFA P2Y results in the normal and intermediate phenotype subgroup. Any discrepancy in platelet function tests was confirmed by repeat P2Y with platelet aggregation to confirm findings.

Concurrent intake of PPI can interfere with clopidogrel function and hence was noted for each patient and efforts made to test at trough PPI times.

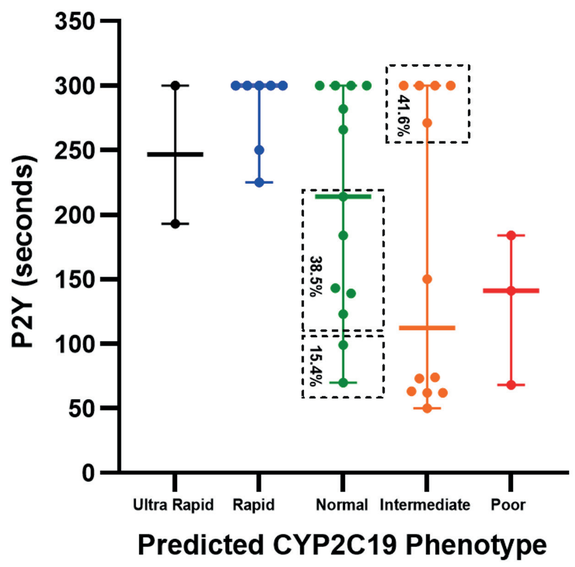

Figure 1. CYP2C19 phenotype against P2Y platelet function test (n=37).
Discordant platelet function results highlighted in dashed areas with corresponding percentages.
15.4% of predicted normal metabolizers (⁎1/⁎1) there is no inhibition of the P2Y12 receptor (P2Y <100 seconds), and 38.5% showed mild inhibition only.
The P2Y results for predicted intermediate phenotypes (⁎1/⁎2, ⁎2/⁎17) were spilt with 41.6% showing complete inhibition of the P2Y12 and the rest showing no inhibition.

Discussion

1. Correlation between Genedrive® POCT and lab-based genetics was excellent where both platforms identified the same CYP2C19 genotypes with only one discordant result was identified, a rare ⁎35 allele not detected in the xTAG CYP2C19 kit. Clinically not significant, though an interesting find as testing increases additional rare alleles will be identified. There are 351 possible diplotype combinations for CYP2C19 gene,[4] awareness and clinical implications with regards to LOF will require constant evaluations and data gathering.

2. The relationship of the CYP2C19 genetic results and platelet function assessment correlated well in the ultra rapid, rapid and poor CYP2C19 phenotype groups, however, the normal and intermediate group required further examination to determine actually physiological impact.

3. The predicted normal metaboliser group have two functioning alleles and are expected to show complete P2Y inhibition. The discordant results shown in our data between predicted CYP2C19 phenotype and P2Y functional assessment highlight that other variables such as PPI intake, raised VWF, body mass index and date of thrombotic event could also be impacting efficacy of clopidogrel and that the metabolism of clopidogrel is not solely dependent on a patients CYP2C19 genotype. The use of PPIs, particularly omeprazole, and their reduction of clopidogrel efficacy has been documented previously[5]. Further investigation is required to evaluate the use of PPIs, specifically lansoprazole alongside clopidogrel with regards to each predicted CYP2C19 phenotype.

4. For the intermediate metabolizer group where one allele is normal, 41.8% showed complete inhibition of P2Y12 receptor. Intermediate metabolisers are advised to avoid clopidogrel if possible due to the LOF of one allele, however as shown, the LOF of one allele does not cause a reduction of clopidogrel metabolism in all predicted intermediate metabolisers.

5. The administration or cessation of clopidogrel cannot be determined by CYP2C19 genetic analysis alone and thus we have proposed a new testing algorithm detailed in Figure 2.

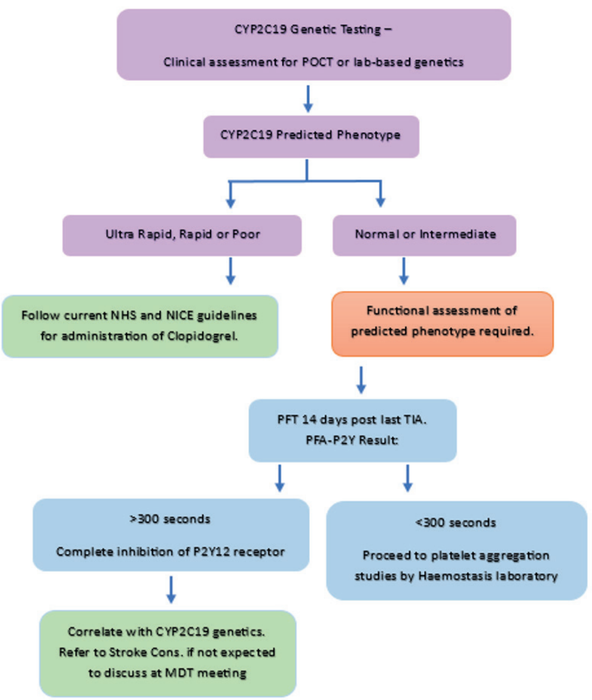

Figure 2. Proposed clopidogrel resistance algorithm formulated from the predicted CYP2C19 phenotype via P2Y data evaluation.

Conclusion

- The Genedrive® POCT and lab-based genetics for analysis of CYP2C19 genotype are in agreement with a 97% correlation.
- In certain instances CYP2C19 genotype and predicted phenotype results require additional platelet function testing to confirm P2Y12 receptor inhibition.

References

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