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Clopidogrel resistance in stroke patients (The CRISP Trial)

Abstract

Objective: To investigate the incidence of clopidogrel resistance in patients with acute ischaemic stroke and to evaluate whether there is an association between clopidogrel resistance and the occurrence of a further cerebrovascular ischaemic event using the vasodilator-stimulated phosphoprotein (VASP) index as a marker of clopidogrel resistance.

Methods: It is a prospective cohort study that recruited 120 patients from the acute stroke unit at the Royal Liverpool University Hospital. All patients with confirmed acute ischaemic stroke had clopidogrel 75mg/day at discharge or after 14 days of acute stroke if deemed by the direct clinical team to be the most appropriate treatment. After at least 7 days of clopidogrel 75mg/day, all those patients fulfilling inclusion/exclusion criteria had phosphorylation of vasodilator-stimulated phosphoprotein (VASP) measured. If VASP measured ≥50% after ≥7 days of clopidogrel maintenance, these patients were deemed as 'clopidogrel resistant', while those with VASP <50% were deemed as 'clopidogrel responder'. Statistical analysis was by univariable analysis which considered the association of each variable – diagnosis, age, duration of clopidogrel, VASP, days to VASP, and number of comorbidities – with the outcome. Risk of second stroke after a first at 6, 12 and 24 months was estimated using logistic regression.

Results: No variables were significantly associated with risk of stroke at 6 months with clopidogrel resistance having no significant effect on likelihood of a further stroke compared to the no clopiodgrel resistance cohort (p value= 0.39). Results were similar at 12 months follow up. However, at 24 months VASP index was significantly associated with risk of a further stroke; each one unit increase in VASP was associated with a 3% increase in risk of stroke at 24 months (p value = 0.05, CI Interval of 1.00- 1.06).

Conclusion: No variables were significantly associated with risk of further stroke at 6 months and 12 months after a first stroke. However, VASP was significantly associated with risk of further stroke at 24 months with increasing VASP leading to a higher risk of further stroke.

Keywords: clopidogrel resistance, ischaemic stroke, vasodilator-stimulated phosphoprotein

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Introduction

Clopidogrel is a thienopyridine compound with ADP-receptor antagonising effects. Current evidence advocates its use in secondary prevention of ischaemic stroke. However, studies have demonstrated that clopidogrel's antiplatelet effect is not uniform in all patients and inter-individual response in platelet response to clopidogrel is known to be large.[1–5] Many patients on clopidogrel after acute ischaemic stroke continue to sustain further cerebrovascular ischaemic events which are associated with significant mortality and morbidity.

The concept of clopidogrel resistance has emerged in medical literature to reflect the failure to inhibit platelet function in vitro, though its existence and definition remains to be more fully established. It has been proposed that the term resistance encompasses patients for whom clopidogrel does not achieve its full pharmacological effect, and failure of therapy reflects patients who have recurrent events on therapy.[6]

Clopidogrel has been used far earlier to reduce the incidence of recurrent ischaemic events in patients with acute coronary syndrome[7] and after coronary stenting.[8] In this patient group, a significant number of cardiovascular recurrences has long been identified[7,8] and owing to this, clopidogrel resistance has been more extensively analysed. Poor response or resistance to clopidogrel represents 10% and 40% of patients with acute coronary syndrome receiving therapy, depending on the tests and thresholds used.[2,9,10] Several methods have been developed to deal with clopidogrel resistance of which the most popular strategy is increasing the loading dose.[11–17] In order to find a better method to tackle clopidogrel resistance, Bonello et al adjusted the clopidogrel loading dose according to platelet monitoring using the vasodilator-stimulated phosphoprotein (VASP) index in a multi-center randomised prospective study, and observed that it was safe and significantly improved the clinical outcomes in patients with clopidogrel resistance.[18]

However, there is a paucity of studies demonstrating the incidence and clinical relevance of clopidogrel resistance in patients with acute ischaemic stroke. One study evaluated the rate of clopidogrel resistance in patients with ischaemic stroke after taking 75mg/day for >/= 1 week as 8.1% and 18.1%, depending on the measurement methods and concentration of the platelet aggregation agent used.[19]

Aim

To investigate the incidence of clopidogrel resistance in patients with acute ischaemic stroke and to evaluate whether there is an association between clopidogrel resistance and the occurrence of further cerebrovascular ischaemic events using VASP.

Methodology

Study design: A prospective cohort study recruiting 120 patients from the acute stroke unit at the Royal Liverpool University Hospital. Patients were recruited to the study during admission to the Acute Stroke Unit during March 2013 to March 2016 while they had further assessment and investigation.

All patients with confirmed acute ischaemic stroke had clopidogrel 75mg/day at discharge or after 14 days of acute stroke if deemed by the direct clinical team to be the most appropriate treatment. After at least 7 days of clopidogrel 75mg/day, all those patients fulfilling inclusion/exclusion criteria had phosphorylation of vasodilator-stimulated phosphoprotein (VASP) measured. If VASP measured ≥50% after ≥7 days of clopidogrel maintenance, these patients were deemed as 'clopidogrel resistant', while those with VASP <50% were deemed as 'clopidogrel responder'. The blood sample was drawn by the patient's bedside if still an in-patient. If the patient had been discharged they were asked to return to the research office where a member of the research team trained in phlebotomy was able to obtain the sample. Travel costs were reimbursed to the patients if required.

Both patient groups were followed up for 24 months to ascertain the recurrence of cerebrovascular ischaemic events. This involved either a brief telephone call to the patient or discussion with their GP by the research team to assess whether or not they have had further cerebrovascular ischaemic events.

Study population

Stroke patients admitted to the Acute Stroke Unit at the Royal Liverpool University Hospital. This also included patients who have undergone other treatments including thrombolysis.

Inclusion criteria

1. Acute Ischaemic stroke or Transient Ischaemic Attack (diagnosed made and/or confirmed by a Stroke Physician)
2. Patients who will be started on clopidogrel 75mg/day during their admission or on discharge.

Exclusion criteria

1. Haemorrhagic stroke or haemorrhagic transformation of recent infarction
2. Patients who were on warfarin at presentation and had a therapeutic INR and/or those who would require anticoagulation with warfarin within 6 months of admission (Paroxysmal Atrial Fibrillation, Mechanical heart valve, Thrombophilia, Cardiac thrombus, DVT/PE, etc). Note: Patients who were on warfarin prior to admission and in whom warfarin was discontinued - for at least 24 months after admission - could be included in the study)
3. Evidence of significant carotid artery stenosis on imaging not considered for endarterectomy
4. Contraindication to platelet therapy (platelet count <100, history of severe bleeding diathesis)
5. Severe strokes with expected survival of <12 months
6. Concurrent severe illness with expected survival of <12 months

VASP calculation

VASP index phosphorylation analysis was performed within 24 hours of blood collection by an experienced technician using platelet VASP kits (Diagnostica Stago, Asnières – France). A citrated blood sample was incubated with Prostaglandin E1(PGE1) or with PGE1 and ADP 10µmols/L for 10 minutes and fixed with paraformaldehyde, after which platelets were permeabilised with nonionic detergent. Analyses were performed on an EPICS XL-MCL flow cytometer (Beckman Coultronics, Margency – France), the platelet population was identified by its forward and side scatter distribution, and 10,000 platelets were gated. A PRI VASP was calculated from the median fluorescence intensity (MFI) of the samples incubated with PGE1 or PGE1 and ADP using the formula: PRI VASP index = [(MFIPGE1 - MFIPGE1+ADP)/MFIPGE1] x 100.

Statistical analysis

Categorical variables were summarised with counts and percentages. Normality of continuous variables was assessed by visual inspection of the histogram for that variable. Normally distributed continuous variables summarised with means and standard deviations while skewed continuous variables summarised with medians and interquartile ranges.

Risk of a second stroke after a first at 6, 12 and 24 months was estimated using logistic regression. The association of each variable with the outcome was considered univariably. The list of variables was chosen by clinical consensus: diagnosis, age, duration of clopidogrel, VASP, days to VASP and number of comorbidities.

Results

Patient characteristics are summarized in Table 1.

Risk of stroke at 6, 12, and 24 months according to each variable of clinical interest, can be seen in Table 2. No variables were significantly associated with risk of stroke at 6 or 12 months. At 24 months, VASP was significantly associated with risk of stroke at 24 months. In particular, each 1 unit increase in VASP was associated with a 3% increase in risk of stroke at 24 months.

According to the VASP measurements, there were fewer patients with Clopidogrel Resistance (CR), (n= 13, Table 1) than patients who were Not clopidogrel resistant (NCR) (n= 103, Table 1). LACS stroke was found to be higher in the CR cohort (n= 5, Table 1), compared to the NCR cohort, where PACS was higher (n= 35, table 1). The median duration of clopidogrel in patients in CR group (n= 26, Table 1) was shorter than the NCR group (n= 36, Table 1). Higher percentage of patients in the CR cohort had 4 co- morbidities (39%) compared to NCR where majority of patients had 1co- morbidity.

Table 1 Patient characteristics

Variable, n (%) unless otherwise stated	VASP resistant (n=13)	Not VASP resistant (n=103)	Total (n=116)
Age (years) at first stroke, median (interquartile range)	70 (69, 75)	67 (60, 77)	69 (60, 77)
Primary diagnosis			

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Table continued...

Variable, n (%) unless otherwise stated	VASP resistant (n=13)	Not VASP resistant (n=103)	Total (n=116)
LACS	5 (38)	31 (31)	36 (32)
PACS	3 (23)	37 (36)	40 (35)
POCS	0 (0)	8 (8)	8 (7)
TACS	1 (8)	8 (8)	9 (8)
TIA	4 (31)	17 (17)	21 (18)
Missing	0	2	2
Duration of clopidogrel, median (interquartile range)	26.0 (16.0, 62.0)	36.0 (26.5, 57.5)	35.0 (24.0, 59.3)
Days until VASP, median (interquartile range)	40.0 (32.0, 63.0)	42.0 (32.5, 55.5)	41.50 (32.0, 57.3)
Number of Comorbidities			
0	2 (15)	9 (9)	11 (9)
1	2 (15)	40 (39)	42 (36)
2	1 (8)	28 (27)	29 (25)
3	4 (31)	16 (15)	20 (17)
4	3 (23)	7 (7)	10 (9)
5	1 (8)	2 (2)	3 (3)
6	0 (0)	1 (1)	1 (1)
6 month outcomes			
Stroke			
No	12 (92)	100 (97)	112 (97)
Yes	1 (8)	3 (3)	4 (3)
TIA			
No	13 (100)	101 (98)	114 (98)
Yes	0 (0)	2 (2)	2 (2)
12 month outcomes			
Stroke			
No	12 (92)	100 (97)	112 (97)
Yes	1 (8)	3 (3)	4 (3)
TIA			
No	13 (100)	102 (99)	115 (99)
Yes	0 (0)	1 (1)	1 (1)
24 months outcomes			
Stroke			
No	11 (85)	101 (98)	112 (97)
Yes	2 (15)	2 (2)	4 (3)
TIA			
No	13 (100)	99 (96)	112 (97)
Yes	0 (0)	4 (4)	4 (3)

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Table 2 Univariable results for risk of a second stroke after a first at 6, 12 and 24 months

Values in italics are for model intercepts		**6 months**		**12 months**		**24 months**	
Variable	Categories	Odds Ratio (95% Confidence Interval)	p-value	Odds Ratio (95% Confidence Interval)	p-value	Odds Ratio (95% Confidence Interval)	p-value
Primary diagnosis	Intercept	-3.69	<0.001	-2.97	<0.001	-2.97	<0.001
	PACS	1	-	1	-	1	-
	LACS	1.11 (0.07, 18.42)	0.94	1.11 (0.15, 8.34)	0.92	0.54 (0.05, 6.23)	0.62
	POCS/TACS	5.33 (0.45, 63.22)	0.18	0.00 (0.00, ∞))	1	0.00 (0.00, ∞)	1
	TIA	0.00 (0.00, ∞)	0.99	0.00 (0.00, ∞)	1	0.97 (0.08, 11.41)	0.98
VASP		-3.33	<0.001	-3.31	<0.001	-4.66	<0.001
		1.00 (0.97, 1.03)	1	1.00 (0.97, 1.03)	0.95	1.03 (1.00, 1.06)	0.05
Age (years)		-5.81	0.1	-2.97	0.32	-4.82	0.15
		1.04 (0.94, 1.14)	0.47	0.99 (0.91, 1.08)	0.9	1.02 (0.93, 1.12)	0.65
Duration of clopidogrel		-3.31	<0.001	-3.31	<0.001	-2.04	0.08
		1.00 (1.00, 1.00)	0.89	1.00 (0.97, 1.03)	0.76	0.96 (0.90, 1.04)	0.32
Days to VASP		-4.36	<0.001	-3.46	<0.001	-2.56	0.02
		1.02 (1.00, 1.04)	0.11	1.00 (0.98, 1.03)	0.86	0.98 (0.94, 1.03)	0.49
Number of comorbidities		-4.36	<0.001	-4.39	<0.001	-4.74	<0.001
		1.06 (0.49, 2.28)	0.88	1.59 (0.79, 3.18)	0.19	1.80 (0.90, 3.61)	0.1

Discussion

The majority of previous studies examining Clopidogrel resistance have focused on patients with cardiovascular disease. By contrast, less is known about the response to clopidogrel in patients suffering from ischemic stroke. After following patients from their first stroke, after 6 months, 8% of patients had a stroke in the CR group which was clinically greater compared to the NCR group of 3%. However, applying univariable analysis no variables were significantly associated with risk of stroke at 6 months (Primary diagnosis, VASP index, Resistance/ Not resistant to Clopidogrel, Duration of Clopidogrel, amount of days VASP measurement calculated and number of co- morbidities). With Clopidogrel resistance having no significant difference of increased likelihood of a stroke compared to the NCR cohort (p value= 0.39).

These results had a similar outlook as the 12 months follow up. No variables were significantly associated with risk of stroke at 12 months (Primary diagnosis, VASP index, Resistance/ Not resistant to Clopidogrel, Duration of Clopidogrel, amount of days VASP measurement calculated and number of co- morbidities). Similarly as 6 months, Clopidogrel resistance no significant difference of increased likelihood of a stroke compared to the NCR cohort (p Value = 0.39).

However, at 24 months VASP index was significantly associated with risk of stroke at 24 months. When handled as a continuous variable, each 1 unit increase in VASP was associated with a 3% increase in risk of stroke at 24 months (p value = 0.05, CI Interval of 1.00- 1.06). Patients with clopidogrel resistance according to VASP measurement of resistance were 9 times more likely to have a stroke at 24 months than those who were not resistant (p value = 0.03, CI interval of 1.17- 71.78).

Other variables at 24 months (Primary diagnosis, VASP index, Resistance/ Not resistant to Clopidogrel, Duration of Clopidogrel, amount of days VASP measurement calculated and number of co-morbidities) were not clinically significant. In comparison, according to Matetzky et al.[20] study 40% of patients in the clopidogrel resistant group sustained a cardio- vascular event at 6 months (P value = 0.007) according to ADP-induced platelet aggregation. Whereas, our study did not displayed a significant difference for recurrence in stroke in the CR Cohort at follow up at 6 months. However, Matetzky sample size in total was 60 participants, and so results may not be comparable although, Matetzky et al had a larger cohort of CR cohort (n= 15), compared to our CR cohort study (n= 13). Also, Matetzky used a different calculation for measuring clopidogrel resistance using ADP-induced platelet aggregation, whereas our study had used VASP, so the measurement of resistance to clopidogrel may differ.

A study by Bonello et al.[18] had measured loading dose of clopidogrel according to vasodilator- stimulated phosphoprotein (VASP) index in patients with clopidogrel resistance undergoing percutaneous coronary intervention (PCI). Which had shown eight major adverse cardiac events (5%) were noted after 1 month follow up, compared to the VASP- guided clopidogrel loading patients (0% vs. 10%; p = 0.007). This study had a large number of cohort with patients with clopidogrel, however, there was no control group (NCR) in this study, to compare outcomes of cardiac events 1 month; this could have inferred bias. In light of these studies, cardiovascular events were more likely to happen at a shorter period of time, compared to patient's outcome of a stroke (24 months; Odd 9.18 p= 0.03). Possible implications, for this study shows that clopidogrel resistance and risk of recurrence of stroke has a longer term effect. VASP measurement can be delayed until 24 months and these patients should be followed up in 24 months time and possibly have a readjustment of their dose/ use a different anti- platelet agent before 24 months.

However, our study limitations involves a small sample size especially our CR group (n= 13), compared to NCR group, and this may have led to Type 2 error in the 6 and 12 months follow up. This study was based on one centre (Royal Liverpool University Hospital) and so this study results may not reliably applied to centres outside our study's centre, hence a larger cohortt with multi- centre approach

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to confirm our results. We did not include a method of reassuring compliance of clopidogrel intake and this study does not take this variable into account for outcomes on further recurrence of stroke. Multivariable model fitting was not possible due to the small patient group and the amount of missing data. Whilst multiple imputation could have been used to account for this, the percentage of missingness was too great to justify this approach.[21]).

To help further this research and knowledge of VASP index and Clopidogrel resistance in stroke patients. Using the VASP index to measure loading doses of clopidogrel in stroke patients with clopidogrel resistance comparing outcomes of CR patients who did not have a loading dose of clopidogrel measured according to the VASP Index and following up outcomes of recurrence of stroke at 6 months, 12 months and 24 months.

Larger studies across multiple centres is needed, and a survival analysis approach to modelling the data; modelling time to the stroke rather than the occurrence of stroke at a fixed time point.

Ethical consideration

Prior to commencing this study permission from the ethics committee was sought. This study conforms with ICH-GCP guidelines and local ethical and legal requirements. Written informed consent was from patients to facilitate recruitment to the study. Patient who were unable consent due to pre-existing cognitive impairment or due to residual effects of the sustained stroke were excluded.

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None.

Conflicts of interest

The authors declare no conflicts of interest.

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