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PLATELET RESISTANCE TO ANTI-AGGREGATING EFFECT OF CLOPIDOGREL IN SOLID TUMOR PATIENTS

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Summary

The anti-aggregating effect of clopidogrel (75 mg/day) on platelet function was monitored in 33 solid tumor patients. Clopidogrel irreversibly blocks the platelet $P2Y_{12}$ receptor and inhibits platelet aggregation induced by adenosine diphosphate (ADP). Whole blood aggregation was measured using a Siemens PFA-100 aggregometer including the Innovance PFA P2Y cartridge. The mean age of patients was 62 ± 13 years. Among them, there were 19 (58%) males and 14 (42%) females. The values of platelet aggregation >106 seconds and <106 seconds showed the anti-aggregating effect of clopidogrel in 22 (67%) and no effect (no response) in 11 (33%) patients, respectively. The average anti-aggregating effect of clopidogrel was 219 ± 110 seconds. No differences in platelet aggregation between males and females were observed (p=0.784). The interindividual variation in platelet aggregation was 50%. There was no statistically significant difference between two measurements on 7 samples of the same individuals performed at an interval of a month or more, showing the intraindividual stability of clopidogrel activity on platelet aggregation (p=1.000).

KEYWORDS: *tumors, clopidogrel resistance, PFA-100 aggregometer, Innovance PFA P2Y*

TROMBOCITNA REZISTENCIJA NA KLOPIDOGRELSKI ANTIAGREGACIJSKI UČINAK U BOLESNIKA SA SOLIDNIM TUMORIMA

Sažetak

Praćen je klopidogrelski (75 mg/dan) antiagregacijski učinak na trombocite u 33 bolesnika sa solidnim tumorima. Klopidogrel ireverzibilno blokira trombocitni receptor $P2Y_{12}$ i sprječava trombocitnu agregaciju pokrenutu adenozin difosfatom (ADP). Agregacija je mjerena u punoj krvi agregometrom Siemens PFA-100 s uloškom Innovance PFA P2Y. Prosječna dob bolesnika bila je 62 ± 13 godine. Muškaraca je bilo 19 (58%), a žena 14 (42%). Vrijednost trombocitne agregacije >106 sekunda pokazala je klopidogrelski antiagregacijski učinak u 22 (67%) bolesnika, a vrijednost <106 sekunda uočena je u 11 (33%) bolesnika koji nisu reagirali. Prosječni klopidogrelski antiagregacijski učinak bio je 219 ± 110 sekunda. Agregacijskih razlika između muškaraca i žena nije bilo (p=0,784). Interindividualna agregacijska varijacija bila je 50%. Nije bilo statistički značajne razlike između dvaju mjerenja 7 uzoraka istih osoba u razmaku od mjesec ili više dana, što pokazuje intraindividualnu stabilnost klopidogrelskog djelovanja na trombocitnu agregaciju (p=1,000).

KLJUČNE RIJEČI: *tumori, rezistencija na klopidogrel, PFA-100 agregometar, Innovance P2Y*

INTRODUCTION

Adenosine diphosphate (ADP) is important for platelet function. Three ADP receptors have been identified: $P2Y_1$, $P2Y_{12}$ and $P2X_1$. Stimulation of the $P2Y_{12}$ receptor can lead to irreversible platelet aggregation and the development of blood clots (1-3). Approximately 15% of clopidogrel is

metabolized by cytochrome P450 3A4 (CYP3A4) liver enzyme, and about 85% is excreted in the stool (4-6, 8). Its metabolites bind to the $P2Y_{12}$ receptor and inhibit ADP-induced aggregation (7). Resistance to clopidogrel can be due to P450 3A4 polymorphisms, and polymorphisms of the platelet $P2Y_{12}$ receptor. The reduced effect of clopidogrel can be due to irregular drug intake, inappropriate dosage or drug interactions, poor absorption, and variable conversion to its active metabolite or increased clearance of the active metabolite.

The anti-aggregating effect of clopidogrel has a normal, bell-shaped Gaussian distribution, which means that some people develop a weaker and others a stronger response to the same dosage (9). Resistance to clopidogrel may be a marker of an increased risk for a thrombotic event.

MATERIAL AND METHODS

Blood samples were collected from patients with cancer who, for their blood circulation problems, were taking clopidogrel anti-aggregation therapy at a dose of 75 mg/daily.

During 18 months, 33 such patients were registered, of whom 19 (58%) were male and 14 (42%) were female (Fig. 1). The mean age of patients was 62 ± 13 years (Fig. 2). The intraindividual stability of aggregation parameters was tested in the same 7 patients whose blood was taken on two occasions, at an interval of a month or more. Blood was collected in 4.5 mL glass Vacutainer blood collection tubes with 0.105 molar buffered sodium citrate (3.2%). Platelet aggregation was measured in whole blood using the Siemens PFA-100 System (Platelet Function Analyzer) including the Innovance PFA P2Y cartridge. Clopidogrel prolongs the aggregation time. The analyzer and reagent manufacturer has set the reference value for clopidogrel aggregation at 106 seconds, which was taken as the cut-off for the purpose of this study. Results below the value were assumed to be normal or showing no effect of clopidogrel, and were labeled as clopidogrel resistant. The obtained results were statistically processed using the Student's t-test and Mann-Whitney Rank Sum Test to compare mean values (x), standard deviations (sd), coefficient of variation (CV), minimum (min) and maximum (max).

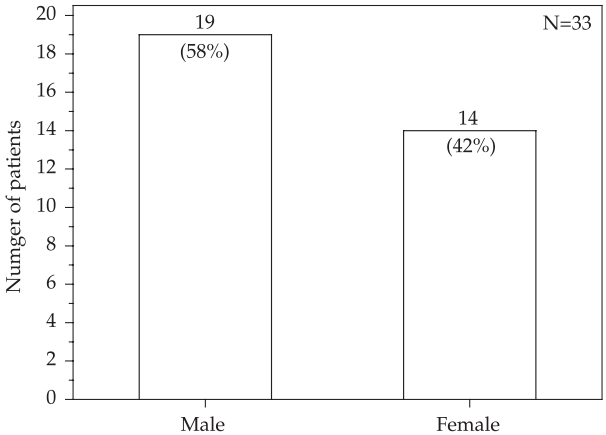

Figure 1. Representation of patients by gender. Out of 33 patients, 19 (58%) were male, and 14 (42%) were female (N = number of patients)

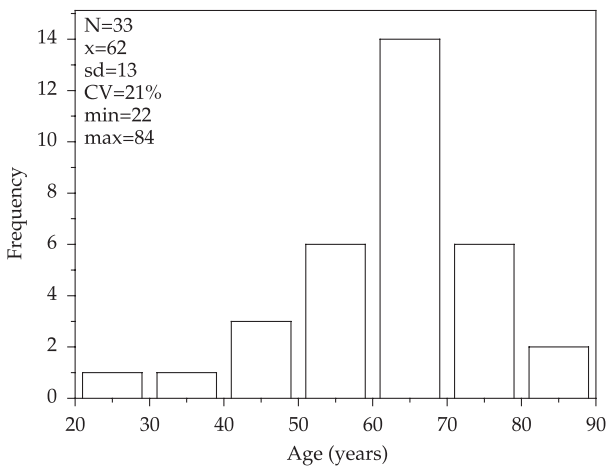

Figure 2. Patient distribution by age. The mean age of 33 patients was 62 ± 13 years; age variability = 21%. The youngest patient was 22, and the oldest one was 84 years of age. (N = number of patients, x = mean value, sd = standard deviation, CV = coefficient of variation, min = minimum, max = maximum).

RESULTS

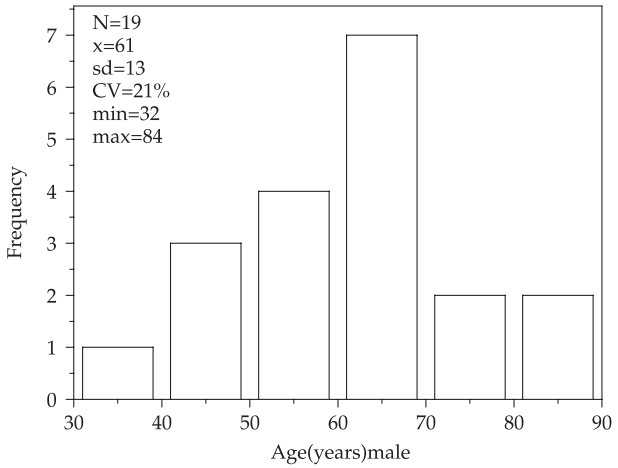

Figure 3. Age-adjusted distribution of male patients. The mean age of 19 male patients was 61 ± 13 years; age variability = 21%. The youngest male patient was 32, and the oldest one is 84 years of age. (N = number of patients, x = mean value, sd = standard deviation, CV = coefficient of variation, min = minimum, max = maximum).

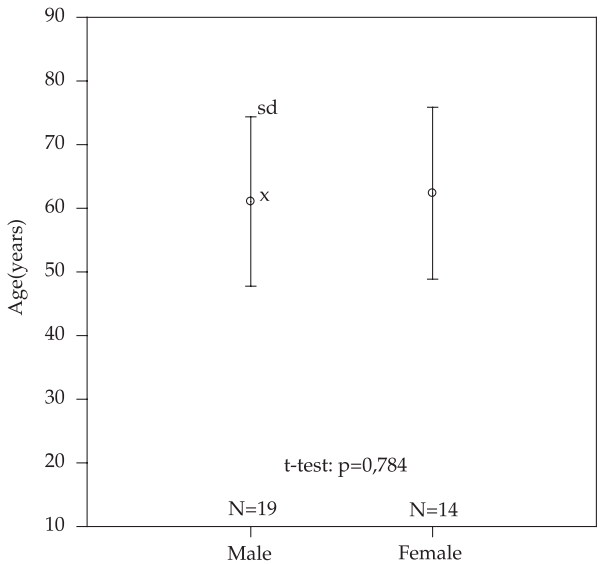

Figure 5. Comparison between age and gender groups. There is no statistically significant age-related difference between males and females (t-test: p=0.784). (N = number of patients, x = mean value, sd = standard deviation, p = probability).

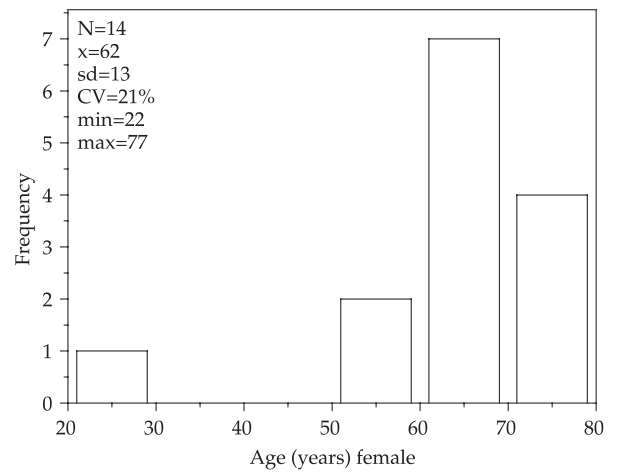

Figure 4. Age-adjusted distribution of female patients. The mean age of 14 female patients was 62 ± 13 years; age variability = 21%. The youngest female patient was 22, and the oldest one was 77 years of age. (N = number of patients, x = mean value, sd = standard deviation, CV = coefficient of variation, min = minimum, max = maximum).

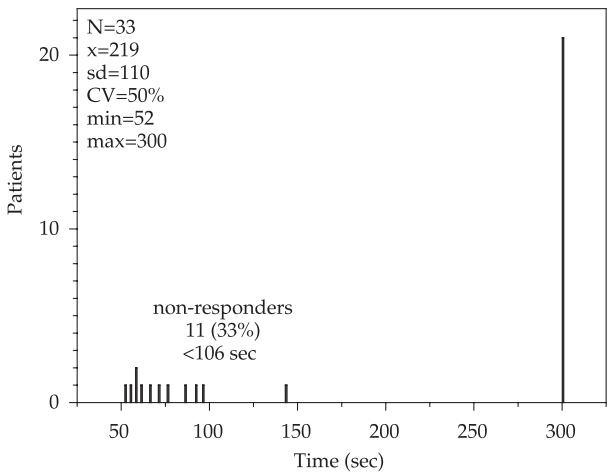

Figure 6. Distribution of platelet aggregation times. Out of 33 patients, 11 (33%) had platelet aggregation time <106 sec, showing no response to clopidogrel therapy. The mean platelet aggregation time in 33 patients was 219 ± 110 sec; aggregation variability = 50%. The lowest and the highest aggregation values were 52 sec and 300 sec, respectively.

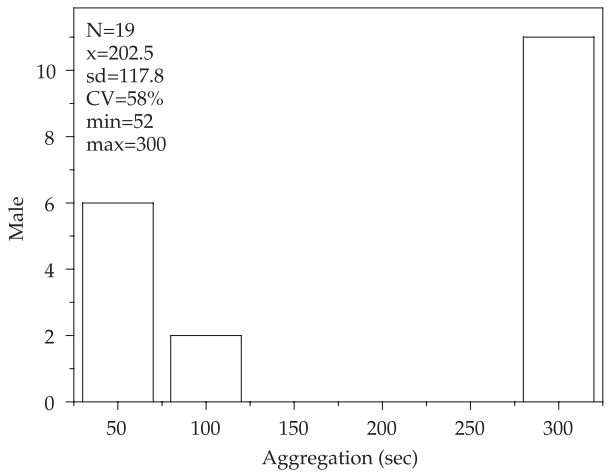

Figure 7. Distribution of platelet aggregation times in male patients. The mean platelet aggregation time in 19 male patients was 202 ± 118 sec; aggregation variability = 58%. The lowest and the highest aggregation values were 52 sec and 300 sec, respectively.

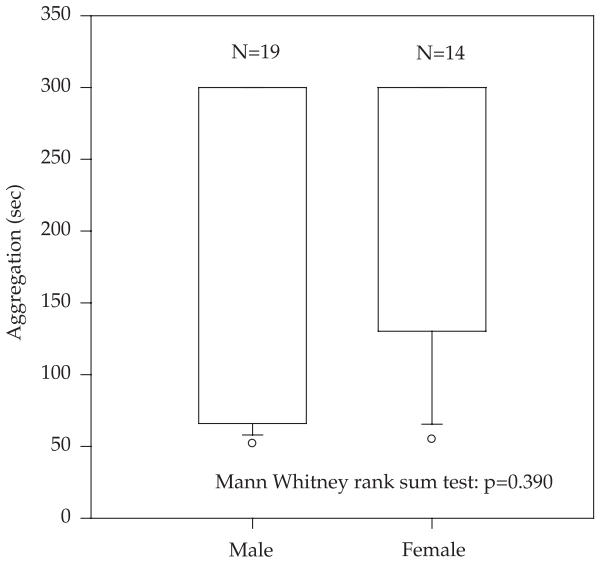

Figure 9. Comparison between platelet aggregation time and gender groups. There is no statistically significant aggregation time-related difference between male and female patients on clopidogrel therapy (Mann-Whitney rank sum test: p=0.390).

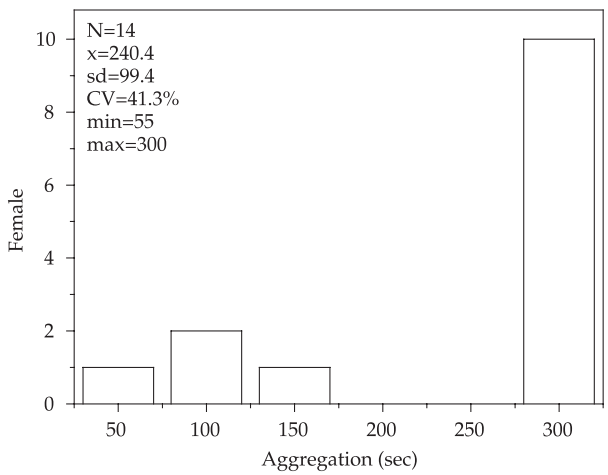

Figure 8. Distribution of platelet aggregation times in female patients on clopidogrel therapy The mean platelet aggregation time in 14 female patients was 240 ± 99 sec; aggregation variability = 41%. The lowest and the highest aggregation values were 55 sec and 300 sec, respectively.

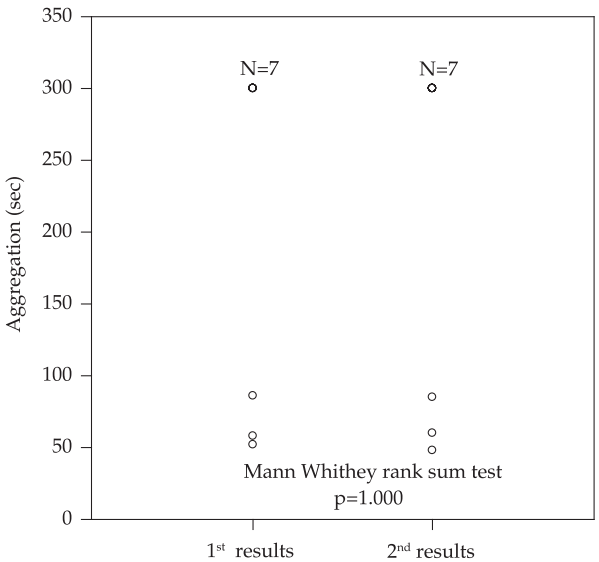

Figure 10. Comparison between two aggregation measurements from the same individuals. The measurements were performed at an interval of a month or more. There was no statistically significant measurement between the first and the second measurement (Mann-Whitney rank sum test: p=1.000). The platelet aggregation time in patients on clopidogrel therapy showed the intraindividual stability in the time pattern.

DISCUSSION

In our previous study, we found platelet aggregation resistance to acetylsalicylic acid (ASA) in about 60% of study subjects (11). In this study, 33% resistance to clopidogrel anti-aggregation therapy was shown. Measurements performed by other authors using a different method, i.e. platelet aggregation flow cytometry, showed the cut-off value for clopidogrel platelet aggregation of 15%. Measurements of platelet aggregation using a simple test such as the VerifyNow-P2Y12 test demonstrated a poor response in 20% of study subjects (12). Also, some additional studies showed 20% resistance to clopidogrel (13-15). The Vasodilator-Stimulated Phosphoprotein (VASP) phosphorylation assay and the VerifyNow-P2Y12 31 test revealed resistance to clopidogrel of 29% and 6%, respectively (16). Clopidogrel resistance variability, as shown by other authors, ranges from 5-63% (16-24). Our result of 33% clopidogrel resistance conforms to average results of worldwide measurements.

CONCLUSION

The metabolites of clopidogrel irreversibly block the platelet P2Y12 receptor and inhibit the binding of ADP to this receptor, resulting in the absence of platelet aggregation. It is important to know that there is a certain proportion of people who do not respond to a clopidogrel dose of 75 mg/day and are therefore at an increased risk of developing thrombosis. Our results showing 33% resistance to the effects of clopidogrel corroborate this fact. The clopidogrel effect on platelet aggregation is found to be slightly greater in women than in men, but the difference is not statistically significant. The effect of clopidogrel remains constant over time for each individual. Regardless of the method for measuring platelet aggregation, the patient receiving clopidogrel for the risk of thrombotic events needs extra monitoring and appropriate clopidogrel dosing to avoid the impact of drug resistance.

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