

Diagnostic accuracy of mean platelet volume in prediction of clopidogrel resistance in patients with acute coronary syndrome

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ABSTRACT

Objective: Clopidogrel therapy is the standard of care in patients with acute coronary syndrome (ACS) and stent implantation. However, concern arises because 25% of subjects are nonresponders to clopidogrel. As this nonresponsiveness is associated with increased adverse outcome, detection of these subjects in daily practice is important in order to withhold a more aggressive therapy and closer follow up. In this study we aimed to evaluate the relation between mean platelet volume (MPV) which is an indicator of platelet activation and clopidogrel nonresponsiveness.

Methods: The study was planned as a prospective cohort study. A total of 185 patients who had been on clopidogrel therapy for any acute coronary syndrome were enrolled in this study. Clopidogrel responsiveness was analyzed by Multiplate MP-0120 device by using the method of whole blood aggregometry. Blood samples were drawn 3.5 days after clopidogrel loading dose. The amount of ADP induced platelet aggregation was assessed as area under curve (AUC), and a cut-off value of 500, above which the patient is considered as clopidogrel nonresponder, was used. MPV was analyzed from the blood which were sampled at the admission of the patient by using automatic hemocounter. Independent sample t-test, ROC analyses and logistic regression analysis were used in statistical analysis.

Results: Among the 185 patients analyzed 41 were found to be clopidogrel nonresponder (22.1%). Mean MPV was found to be significantly higher in nonresponders compared to responders (8.7 ± 0.82 fL vs. 8.1 ± 0.83 fL, $p < 0.001$). A cut-off value of 8.3 fL for MPV was detected in prediction of clopidogrel nonresponsiveness with a sensitivity of 76.6% and specificity of 68.3% (OR: 6.4; 95% CI 2.9-14.1, AUC: 0.70, $p < 0.001$).

Conclusion: This study showed that MPV can be used as a predictor of clopidogrel resistance in patients with ACS. (*Anadolu Kardiyol Derg 2014; 14: 134-9*)

Key words: clopidogrel resistance, acute coronary syndrome, mean platelet volume, diagnostic value, sensitivity, specificity

Introduction

Platelets play a key role in the pathophysiology of thrombosis after plaque rupture. Plaque rupture occurs spontaneously in patients with acute coronary syndromes (ACS) or may be iatrogenically induced in patients undergoing percutaneous coronary intervention (PCI). Among the multiple mediators of platelet activation ADP plays a pivotal role in platelet activation. ADP-P2Y₁₂ receptor interaction causes sustained activation of glycoprotein (GP) IIb/IIIa receptors leading to stable platelet rich thrombus formation at the site of vessel wall injury (1). Therefore clopidogrel whose active metabolite irreversibly inhibits the P2Y₁₂ receptor is a cornerstone of oral antiplatelet therapy in the secondary prevention of coronary artery disease and in

immediate treatment of ACS and PCI. Addition of clopidogrel to aspirin therapy has been associated with better long term clinical outcomes in patients undergoing PCI (2, 3). The long term clinical benefit associated with dual antiplatelet therapy has been also observed in patients with unstable angina and non-ST-elevated myocardial infarction (STEMI) independent of coronary revascularization (4). More recently clinical benefit of clopidogrel has also been extended to patients with STEMI (5, 6). Despite the unambiguous clinical benefit achieved with the adjunct of clopidogrel in ACS/PCI patients a considerable number of patients continue to have cardiovascular events. This has been attributed to variability of platelet response to clopidogrel therapy. Although the mechanism leading to poor clopidogrel effects are not fully elucidated and the best definition to assess



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antiplatelet drug response has not been fully established there is sufficient evidence to support that persistence of enhanced platelet reactivity despite the use of clopidogrel is a clinically relevant entity (7). Multiple studies now have demonstrated a relationship between clopidogrel nonresponsiveness and/or high on treatment platelet reactivity measured by multiple platelet assays and adverse clinical ischemic events (8).

However due to lack of consensus on the optimal methods to quantify high platelet reactivity and the cutoff values associated with clinical risk, the routine measurement of platelet reactivity has not been widely implemented in clinical practice nor recommended in the guidelines. As larger platelets are metabolically and enzymatically more active, and have greater prothrombotic potential, mean platelet volume (MPV) which is a routinely assessed marker is accepted as a potential measure of platelet reactivity (9).

In previous trials elevated MPV has been shown to be associated with other markers of platelet reactivity, and also with increased cardiovascular risk (10, 11). However until now no study exists with a specific purpose of investigating the diagnostic accuracy of MPV test in prediction of clopidogrel resistance. In this study we aimed to determine whether MPV can be used in prediction of clopidogrel hyporesponsiveness in patients with acute coronary syndrome.

Methods

Study design

This study was designed as a prospective cohort study for estimating the diagnostic accuracy of MPV in patients with ACS. For the 185 patients enrolled in the study statistical power of the study was calculated as 0.93.

Study protocol

A total of 301 patients were screened between May 2011 and January 2012 and 185 patients were enrolled in the study. Study participants were consisted of patients who were hospitalized for acute coronary syndrome. Patients with severe anemia, thrombocytopenia, myelodysplastic syndrome, coagulopathy and recent blood transfusion were excluded. In the whole population, clopidogrel was initially started. On admission a loading dose of 300 mg was applied to the patients and this was followed by 75 mg daily dose regimen. In the case of primary PCI the patients were loaded by 600 mg clopidogrel just before the procedure. All of the participants gave written informed consent and the local Research Ethics Committee had previously approved the study protocol.

Study variables

Demographical and clinical variables of the patients were recorded including age, sex, body mass index, diabetes mellitus, hypertension and smoking status. Routine laboratory parameters were also recorded which were consisted of hemoglobin,

total platelet count, MPV, CRP, HDL, LDL, triglyceride, AST, ALT, BUN and creatinine. Creatinine clearance of each patient was calculated by Cockcroft-Gault formula. Concomitant drug therapy of the patients were also recorded.

Assesment of clopidogrel resistance

The blood samples for clopidogrel resistance were drawn 72 hours after the first dose. Clopidogrel resistance was assessed according to ADP induced platelet aggregometry. For this purpose a multiplate electrode aggregometry (MEA) device called Multiplate Analyzer (Dynabyte, Munich, Germany) was used. The instrument analyzes platelet function in whole blood at 37°C by the attachment of platelets on to metal electrodes, leading to a change of the electrical conductivity (or impedance), which is continuously recorded (12). After dilution of hirudin-anticoagulated whole blood and stirring for 3 minutes in the test cuvettes ADP in a final concentration of 6.4 mmol/L (ADP test) were added and aggregation was continuously recorded for 5 minutes. The increase of impedance due to the attachment of platelets to the electrodes is detected and transformed to arbitrary aggregation units that are plotted against time. Aggregation measured with MEA is reported as area under the curve (AUC) of arbitrary units (AU-min) (Fig. 1) (13). The cut-off value for MEA measurements defining the upper quintile (20%) of patients was 500 arbitrary unit (AU) min and 41 patients were therefore defined as clopidogrel low responders (13).

MPV analysis

Samples for MPV analysis were drawn on admission, and analysed within 1 hour after sampling by Beckman Coulter LH 780 Analyzer. The blood samples were stored in EDTA containing tubes.

Clopidogrel resistance measured by MEA was defined as outcome variable and MPV values were defined as predictor variable.

Statistical analysis

Data was presented as numbers and frequencies for categorical variables, and mean±standard deviation or median values for continuous variables. The continuous variables were analyzed for normality, and all of them except CRP were found to have normal distribution. Statistical power of the study was calculated as 0.93 for relation of MPV and clopidogrel response. For comparison between groups, chi-square (or Fisher's exact test when any expected cell count was <5 for a 2x2 table) for categorical variables and independent sample t test or Mann-Whitney U test for continuous variables were applied. A multiple logistic regression analysis was used to identify independent predictors of clopidogrel hyporesponsiveness. Factors entered into the multivariate model were those with p value less than 0.10 from univariate analysis. Thus the independent parameters entered were MPV, CRP, hemoglobin, total platelet count, abnormal liver function tests, nonsmoking, diabetes mellitus and the dependent variable was the clopidogrel resistance. Two-sided p

value less than 0.05 was considered statistically significant. The Pearson correlation coefficient was computed to examine the association between two continuous variables. ROC curve analysis was used for definition of cut-off value for MPV in predicting clopidogrel hyporesponsiveness. The value with highest sensitivity and specificity was assessed as the cut-off value. Statistical tests were performed using SPSS version 15 (SPSS Inc., Chicago, Illinois).

Results

The population characteristics

The mean age of the whole study group was 59.6±11.2. years with a male predominance (80% males vs. 20% females). Of these 185 patients, 31 (16.7%) underwent primary PCI. The great majority of the patients [83%, n=154 received 300mg loading dose while the rest (16.7%, n=31)] received a loading dose of 600 mg.

Platelet aggregometry

At the end of the study, 41 (22.1%) patients were found as hyporesponsive to clopidogrel according to platelet aggregometry. When the whole study population was subgrouped as clopidogrel responders (n=144) and clopidogrel hyporesponders (n=41), the baseline clinical and demographical variables were similar among the subgroups except for total platelet count, C-reactive protein (CRP), hemoglobin level, diabetes mellitus, smoking status and liver function tests (Table 1). Total platelet count (259.926 vs. 228.770 p<0.001) and CRP (23.3 mg/L vs. 8.3 mg/L p<0.001) levels were statistically higher in hyporesponsive group. Whereas hemoglobin levels were significantly lower in hyporesponders (12.7±2.2 vs. 13.5±1.8 g/dL p=0.03) The number of patients with abnormal liver enzymes were also statistically higher in hyporesponsive group (63.4% vs. 47.2% p=0.049). There was significantly less cigarette smokers among hyporesponders when compared with responders (56.1% vs. 74.3% p=0.02). Frequency of diabetes mellitus was significantly higher in hyporesponder group (39% vs. 24.3% p=0.05) As clopidogrel is a pro-drug which is metabolized to its active component via liver enzymes (mainly the CYP2C19 and CYP3A4), drug-drug interactions is also a major cause of clopidogrel hyporesponsiveness. However in our analysis no statistical significance was found in respect of concomitant drug therapies between the hyporesponsive and responsive group including, proton pump inhibitors, statins, ASA, heparin and Gp2b3a inhibitors. The percentages of patients using these drugs were similar in both of the groups (Table 2).

Diagnostic accuracy of MPV

When the patients were analyzed in respect of MPV, the range of MPV was 6.2 fL-11.0 fL with a mean value of 8.25±0.86 fL among the whole study group. The mean MPV was significantly higher in hyporesponsive group compared with responsive group (8.7±0.82 fL vs. 8.1±0.83 fL, p<0.001) (Fig. 1). According to ROC analysis, a cut-off value of 8.3 fL was found to predict clopidogrel hypore-

sponsiveness with a sensitivity of 76.6% and specificity of 68.3%. Odds ratio for this cut-off value was 6.4 with a confidence interval of 2.9-14.1 and p value of <0.001 (Fig. 2). Area under curve of the ROC analysis was found as 0.70 (p<0.001).

In multiple regression analysis, the independent predictors of clopidogrel hyporesponsiveness were found as MPV, CRP, total platelet count and nonsmoking; MPV being the most powerful predictor (OR: 12.1 95% CI 4.2-35.1 p<0.001) (Table 3).

Discussion

Our study is giving evidence for the association between MPV and clopidogrel hyporesponsiveness in patients with acute coronary syndrome. The pretreatment MPV levels which were found significantly higher in clopidogrel hyporesponders can be regarded as a sign of high pretreatment platelet reactivity which is actually one of the major reasons of clopidogrel resistance. ROC analysis and multiple regression analysis showed that high MPV values can be regarded as a predictor of clopidogrel resistance.

Considering the great prevalence of clopidogrel resistance and associated adverse outcomes, early recognition of these patients is very important. However the major problem in this issue is the lack of standardized method and cut-off values in definition of clopidogrel hyporesponsiveness. Several methods have been used but none of these, have been fully standardized or fully agreed upon to measure clopidogrel responsiveness (14). Despite of complicated platelet function tests, this relatively simple and readily available test; measurement of MPV by auto-

Table 1. Baseline clinical and laboratory characteristics of the study population and comparison between the groups

	Clopidogrel hyporesponder group (n=44)	Clopidogrel normoresponder group (n=144)	P
Age-years	61±12.54	59±10.7	0.232
Male, n %	30 (73.2%)	115 (79.9%)	0.238
BMI	27.5±4.1	27.3±3.4	0.78
Hypertension, n %	24 (58.5%)	68 (47.2%)	0.135
Diabetes mellitus, n %	16 (39%)	35 (24.3%)	0.05
LDL-cholesterol, mg/dL	110.2 ±27.1	119.0±35.2	0.14
HDL-cholesterol, mg/dL	34.8±8.8	36.3±9.7	0.39
Triglyceride, mg/dL	164.3±105	165.8±102.6	0.94
Smoking, n %	23 (56.1%)	107 (74.3%)	0.02
Total platelet count	259926.8	228770.8	<0.001
Hemoglobin, g/dL	12.7±2.2	13.5±1.8	0.03
CRP, mg/L*	7.35	3.75	<0.001
Abnormal LFT, n %	26 (63.4%)	68 (47.2%)	0.049
Creatinin clearance, mL/min	92.2±36.1	101.7±30.6	0.10

*median values are given and Mann-Whitney U test is used.
Other numeric values are mean values, chi-square and independent sample t-test are used.
BMI - body mass index; LFT - liver function tests

mated cell counter, has been accepted as a surrogate marker of platelet function (9). Larger platelets are metabolically and enzymatically more active than smaller platelets, containing more prothrombotic material, with increased thromboxane A2 and B2 per unit volume and glycoprotein IIb-IIIa receptor expression (15). They show greater aggregability in response to ADP and decreased inhibition of aggregation by prostacyclin *in vitro* (16). A recent meta-analysis, drawn from 24 studies of over 6000 subjects, supports the hypothesis that elevated MPV is a cardiovascular risk factor that it is associated with adverse cardiovascular events (17). In the only existing literature about correlation of MPV levels and clopidogrel resistance, Huczek et al. (18) showed that MPV levels were strongly correlated with residual platelet reactivity under dual antiplatelet treatment. In this study 36 patients with early stent thrombosis and 72 patients with no stent thrombosis were compared in respect of platelet size and residual platelet reactivity. There was a strong positive correlation between MPV and residual platelet reactivity after treatment, both for ARU (aspirin reaction units) and PRU (P2Y12 reaction units). However in this study the relation between MPV and clopidogrel resistance was assessed only via correlation analysis, and no cut-off value for MPV was defined for prediction of clopidogrel resistance. In our knowledge our study is the first defining a cut-off value for MPV in prediction of clopidogrel resistance. As the range of MPV (6.2 fL-11.0 fL) and prevalence of clopidogrel resistance (22.7%) among whole study group are similar to previous reports, this cut-off value (>8.3 fL) for MPV seems to be a reliable predictor of clopidogrel resistance (OR:6.4 95% CI 2.9-14.1 AUC:0.70 $p<0.001$). However the sum of sensitivity and specificity for MPV in predicting clopidogrel hyporesponsiveness was rather low (147%). Therefore, although MPV could be valuable to predict response to clopidogrel, it should not be used instead of aggregometry. In our analysis variables associated with clopidogrel resistance other than MPV were AMI, diabetes mellitus, liver function abnormality, nonsmoking, high platelet count, low hemoglobin levels and high CRP levels. In multivariate analysis liver function abnormality, AMI, diabetes mellitus and hemoglobin levels were no longer found to be independent parameters of clopidogrel resistance whereas MPV was the most powerful predictor with an odds ratio of 12.1 (OR:12.1 95% CI 4.2-35.1 $p<0.001$). In previous reports inadequate response was shown to be more prevalent in specific patient populations (e.g. ACS, diabetics, overweight) (19, 20). In our analysis there was a trend towards higher prevalence of non-responders among DM (39% vs. 24.3% $p=0.05$). The probable explanation for this association is presence of higher number of immature thrombocytes in diabetic patients (21). Although the higher prevalence of clopidogrel resistance among nonsmokers seems to be a surprising endpoint, this finding was concordant with the previous data. In previous trials, smoking was shown to be a negative risk factor for clopidogrel resistance (22, 23). This was attributed to activation of CYP450 system via the contents of cigarette mainly the nicotine and aromatic hydrocarbons.

Table 2. Comparison of groups in respect of concomitant drug therapy

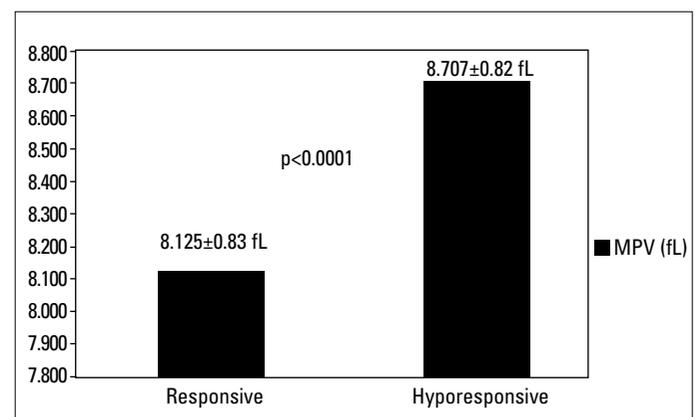
Drug	Hyporesponsive group (n=44)	Normoresponsive group (n=144)	P
Tirofiban	14 (34.1%)	53 (36.8%)	0.453
Heparin	41 (100%)	137 (95.1%)	0.167
PPI	30 (71.1%)	112 (72.6%)	0.681
Beta blockers	33 (80.5%)	121 (84%)	0.373
RAS blockers	40 (97.6%)	136 (94.4%)	0.368
Ca antagonists	4 (9.8%)	13 (9.0%)	0.546
NSAID	11 (26.6%)	35 (23.8%)	0.443
Statin	2 (4.9%)	8 (5.7%)	0.601

NSAID - non-steroidal anti-inflammatory drugs; PPI - proton pump inhibitors; RAS - renin-angiotensin system
Chi-square test is used

Table 3. Multiple logistic regression analysis for independent predictors of clopidogrel hyporesponsiveness

	OR	Lower limit	Upper limit	P
MPV >8,3 fL	12.17	4.209	35.184	<0.001*
CRP mg/L	1.03	1.008	1.061	0.012*
Total platelet count	1.0	1.000	1.000	<0,001*
Nonsmoking	3.75	0.122	0.863	0.024*
Abnormal LFT	1.45	0.281	1.801	0.472
Diabetes mellitus	0.54	0.215	1.391	0.205
HGB	0.93	0.748	1.176	0.579

ACS - acute coronary syndrome; CRP-C - reactive protein; LFT - liver function test; MPV - mean platelet volume
* $p<0.05$

**Figure 1. MPV values in clopidogrel responsive and hyporesponsive group**

Another finding in this analysis was the higher CRP levels in hyporesponder patients (23.3 mg/L vs. 8.3 mg/L $p<0.001$). This significant difference can be attributed to higher concentration of inflammatory mediators associated with higher prothrombotic state mainly via the increased adhesion molecules. Increased CRP levels in clopidogrel hyporesponders was also shown in another study in which $CRP > 2$ mg/L was found to be an independent predictor of high on treatment platelet reactivity (23).

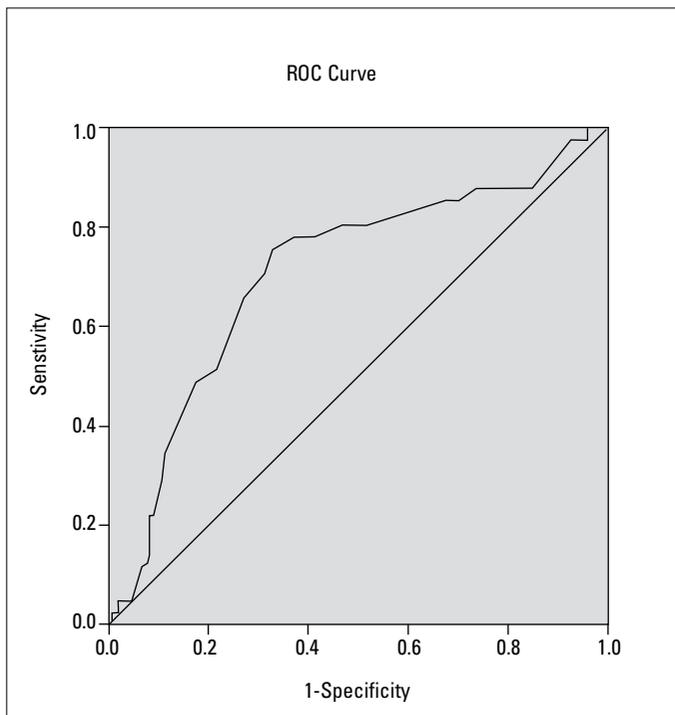


Figure 2. ROC curve analysis of MPV in prediction of clopidogrel resistance

When the association between platelet count and clopidogrel resistance analyzed, we have found a higher platelet count in hyporesponder group which was statistically significant (259.926 vs. 228.770 $p < 0.001$). Most studies found no significant association between increased platelet count and incidence of AMI, restenosis, or long term mortality. Also no relation was found between platelet count and antiplatelet drug response in previous reports (24, 25). Our results are different from the existing literature in respect of this relation. This difference may be due to our method and cut-off point which are based on measurement of absolute number of aggregated platelets in response to ADP rather than the relative difference according to baseline. As expected with increasing total platelet counts, absolute number of aggregated platelets will also increase. In previous reports which have defined no relation between platelet count and high on treatment platelet reactivity, mostly the cut-off point used was $< 10\%$ increase in aggregation in respect to baseline.

Another finding of our study was the relation between liver function abnormality and clopidogrel resistance. The prevalence of abnormal liver functions (defined as liver enzymes above the cut-off value) was significantly higher in hyporesponders compared to responders (63.4% vs. 47.2% $p = 0.049$). As clopidogrel is a prodrug which needs to be activated in liver, this can be regarded as an expected endpoint. The data on this topic mainly focused on existence of genetic polymorphisms of the CYP2C19 and CYP3A4 enzymes which had been found to be closely related to clopidogrel resistance. However liver function abnormality itself, is not considered as a main risk factor for clopidogrel resistance. In a recent case report, Ibrahim et al. (26)

reported a case with right ventricular infarction in whom early stent thrombosis was attributed to clopidogrel resistance which was resolved after the normalization of liver enzymes. In our study although multivariate analysis did not support the predictive value of liver function abnormality in clopidogrel resistance, we think that this can be an important risk factor, as if certain cut-off values for liver enzymes above which the clopidogrel metabolism is effected are determined.

Again considering the metabolism of clopidogrel, many drug-drug interactions have been identified so far, including mainly the proton pump inhibitors, lipophilic statins, some antibiotics and antifungal drugs (27). However in our analysis no significant difference was found between groups in respect of concomitant drugs. This can be attributed to usage of relatively standardized medications for hospitalized patients in our study population.

Study limitations

Our study has some limitations. As long as this is a cross sectional study, no relation could be interpreted on clinical endpoints neither for MPV nor for clopidogrel resistance. Also at this point, we were not able to confirm the cut-off value we used in clopidogrel resistance (upper quintile of our population corresponding to 500AU*min) with clinical end results. Considering the reasons leading to clopidogrel hyporesponsiveness ranging from genetic factors such as polymorphism of the P2Y12-receptor or the CYP3A4-enzyme-system to drug-interactions involving CYP3A4, poor patient compliance, under-dosing, differences in individual absorption and high pretreatment platelet reactivity, the pretreatment MPV values analyzed in this study could represent only the pretreatment platelet reactivity. Measurement of on treatment MPV values could give additional information involving the other mechanisms responsible for poor clopidogrel response. The swelling of the platelets in EDTA containing tubes in a time dependent manner is another possible limitation of our study in respect of MPV measurement.

But in order to overcome this effect we performed the automated blood count analysis within 1 hour after sampling.

Conclusion

Our study is giving evidence for the association between MPV and clopidogrel resistance in patients with acute coronary syndrome. The persistence of this association in this relatively larger patient population is an important finding in routine clinical evaluation of the patients. Considering the great variability of platelet function tests, usage of this simple and already available marker; MPV for this purpose seems to be worthy especially in initial evaluation of the patients. As long as our study is the first identifying a cutoff value for MPV in prediction of clopidogrel resistance, our results if replicated in larger studies could be helpful in guiding the treatment and also the follow up. Also additional data are needed to confirm such a relation for stable angina patients as our study population is not enough to make such an interpretation.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - H.U., E.Ö., Ö.B.; Design - H.U., E.Ö., Ö.B.; Resource - Ö.B., B.A., N.B., Ö.G.; Materials - Ö.B., B.A., N.B., Ö.G.; Data collection&/or processing - H.U., F.A.; Analysis - H.U., E.Ö., Ö.B.; Literature search - H.U., E.Ö., F.A.; Writing - E.Ö., H.U.; Critical review - E.Ö., Ö.B.

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