Development and Validation of Tongue Diagnostic Parameters-Based Diagnostic Signatures in Coronary Artery Disease Patients with Clopidogrel Resistance after Percutaneous Coronary Intervention

Bo Liang

Nanjing University of Chinese Medicine

Cai-Hong Liang

Nanjing Medical University

Jia Lu

Nanjing University of Chinese Medicine

Ning Gu (✉ 20193122@njucm.edu.cn)

Nanjing University of Chinese Medicine <https://orcid.org/0000-0003-0704-6768>

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Abstract

Background: Credible diagnostic stratification remains a challenge for coronary artery disease (CAD) patients with clopidogrel resistance (CR) after percutaneous coronary intervention (PCI). Tongue Diagnostic parameters (TDP)-based diagnostic signatures might predict the diagnostic.

Methods: Clinical and TDP data were obtained from CAD patients with CR after PCI patients and then analyzed. TDP-based diagnostic signatures were developed and validated. Diagnostic prediction was assessed using a receiver operating characteristic (ROC) curve.

Results: A total of 101 patients were consecutively identified. Then, TDP were identified as significantly associated with CR diagnostic and were combined with risk factors to develop a model. ROC curve analysis showed that TDP-based diagnostic signatures performed well in diagnosing CR with an area under the ROC curve value of 0.766.

Conclusions: This study identified and validated a novel TDP-based diagnostic signatures to reliably distinguish CR diagnosis in CAD patients undergone PCI. Further larger, multicenter prospective studies are desired to validate this model.

Introduction

Cardiovascular disease remains a leading cause of morbidity and mortality, despite improvements in outcomes[1, 2]. Age-adjusted coronary artery disease (CAD) mortality has declined since the 1980s, particularly in high-income regions[3]. However, many risk factors, particularly obesity[4] and diabetes mellitus[5], have been increasing substantially. CAD rates are now still high in many countries. Proverbially, percutaneous coronary intervention (PCI) is one of the best treatments for CAD, not just for acute coronary events[6], often with coronary thrombus formation. Although the guidelines don't yet endorse exactly dual antiplatelet therapy duration after PCI[7, 8], it's worth noting that for now, dual antiplatelet therapy is definitely a must. Aspirin and clopidogrel have become integral parts of management in patients with CAD and after PCI. However, the platelet responses to aspirin and clopidogrel are not uniform[9]. Diminished or lack of response to antiplatelet agents has been termed resistance to antiplatelet therapy[10]. The mechanisms of clopidogrel resistance (CR) include alterations in genetic[11–13], pharmacokinetic[14], and platelet properties[15]. There is a dearth of information in regard to the clinical significance, methods to test them, and strategies to treat them[10]. Further research in preference signatures is necessary for early detection, early diagnosis and early intervention appropriately. The recognition of more sensitive and specific signatures for CR diagnosis is required and is expected to result in a better choice of risk-related therapy. The discovery of preference signatures could improve the prognosis of CAD patients with CR after PCI and reduce the burden of constant side effects in surviving individuals.

Traditional Chinese medicine (TCM) has been used in clinical practice for more than two thousand years[16], characterizing whole view and syndrome differentiation, and has shown its unique advantages

in the prevention, treatment, rehabilitation and health care of various diseases[17, 18]. Moreover, Huoxue Huayu therapy, a kind of TCM therapy, is an effective and safe therapy for CAD patients after PCI[19]. However, none of the studies, so far, suggest whether TCM has diagnostic value for CAD patients with CR after PCI. When such an exciting tongue diagnostic parameters (TDP), a characteristic of TCM, is evaluated combined with risk factors as diagnostic signatures, it is highly possible to inspect an association between TDP and diagnostic classification. Here, we scrutinized the diagnostic significance of TDP-based diagnostic signatures for predicting in CAD patients with CR after PCI.

Methods

Study Design

This was a real-world, clinical observation trial conducted at The Affiliated Jiangning Hospital of Nanjing Medical University (Nanjing, China), a large tertiary referral center, to assess the diagnostic value of TDP in CAD patients with CR after PCI. The study was approved by the ethics committee at The Affiliated Jiangning Hospital of Nanjing Medical University (No. 20150221), and patients provided signed written informed consent before enrollment and were able to withdraw from the study at any time and no explanation was required. This clinical trial was performed according to the revised Declaration of Helsinki 2013[20].

Patient Selection

Participants were continuously recruited from May 2015 until December 2016. Patients were eligible if they were adults between the ages of 18 and 75, diagnosed with CAD and undergone PCI. CAD was defined according to 2019 European Society of Cardiology Guidelines[8] or 2019 guiding principles for chronic total occlusion percutaneous coronary intervention[6]. The exclusion criteria were 1) accompanied by hematological diseases (abnormal coagulation, significantly abnormal prothrombin time, and platelet $< 50 \times 10^9/L$ or $> 450 \times 10^9/L$); 2) application of drugs that affect coagulation and platelet function, such as warfarin, within 6 months; 3) rheumatic heart disease, severe valvular disease, pulmonary heart disease, dilated cardiomyopathy, hypertrophic cardiomyopathy, myocarditis and hyperthyroidism; 4) severe anemia, malignant tumors, severe liver and kidney dysfunction (chronic kidney disease $>$ stage 4, Child-Pugh $>$ level C), severe immune system and respiratory diseases, and organ transplantation. Patients who were allergic to clopidogrel or had contraindications and had a history of surgery or major trauma within 3 months were excluded.

Study Products and Procedure

After rigorous inclusion and exclusion criteria, patients were divided into two groups: CR group (CR arm) and control group (Con arm). CR was defined according to previous studies[21–23]. All patients were given loading doses of aspirin (300 mg, Bayer, Germany) and clopidogrel (300 mg, Sanofi, France) before

PCI. All patients received maintenance doses of antiplatelet agents (100 mg/d aspirin and 75 mg/d clopidogrel). All patients were given standardized drug treatment of CAD, combined with other diseases to the corresponding treatment. Thromboelastography (TEG5000, Haemoscope, United States) was performed on the fifth day after PCI. All data were monitored for accuracy.

Identification of Diagnostic Signatures

Clinical data, laboratory examination, TDP determined by TCM Diagnostics[24] and surgical data were potential diagnostic signatures. The TDP included tongue color, tongue form, tongue coating and sublingual complex. Tongue color was measured by tongue colorimetric plate, which was divided into light white, light red, red crimson and indigo blue, corresponding to grades 0, 1, 2 and 3. Tongue form was diagnosed according to size (fat, normal and lean), teeth marks and crack. Tongue coating was composed of color (white, yellow, and dark gray measured by tongue colorimetric plate) and proper (thick and thin coating, smooth, moist and dry coating, greasy coating and exfoliative coating). And sublingual complex was classified into 4 grades according to the degree of stasis: Grade 0, normal; Grade 1, main vein thickened 1/5, slightly bluish color, no obvious dilatation of venous plexus, small nodules, scattered petechiae in sublingual capillaries; Grade 2, main vein thickened 2/5 ~ 4/5, bluish purple color, venous plexus obvious rage, nodules beaded or sugar gourd shape; Grade 3, main vein thickened by more than 1 times, dark purple color, venous plexus obvious rage, nodules distension was treetops and clusters. All patients were observed under natural light from 7:00 a.m. to 8:00 a.m. in a quiet and warm environment, without gargling or drinking stimulating or colored liquid food or drugs by 3 fixed physicians with unified training and TCM internal medicine practice qualification. Then, to uncover the practicability and accuracy of diagnosis signatures for CR, risk factors determined by logistic regression analysis were selected. Moreover, TDP, which were different significantly in arms and performed with χ^2 test, were also analyzed. A two-tailed significance level of $P \leq 0.05$ was considered statistically significant.

Development and Validation of Diagnostic Signatures

Finally, diagnostic signatures were established to build a diagnostic prediction formula, and then validated with 20 patients (including CR and non-CR patients) randomly selected by the double-blind method[17]. The result of thromboelastography was considered as the diagnostic standard. After assessed using a time-dependent receiver operating characteristic (ROC) curve, the area under the ROC curve (AUC) values were computed[25].

Results

Patient Enrollment

Between May 2015 and December 2016, 116 patients were divided into CR arm (43 patients) or Con arm (73 patients) at The Affiliated Jiangning Hospital of Nanjing Medical University. During the study, 11

cases were lost (5 cases in the CR arm and 6 cases in the Con arm, respectively) and 4 cases (1 case in the CR arm and 3 cases in the Con arm, respectively) were excluded because of incomplete data. Finally, 101 cases (37 cases in the CR arm and 64 cases in the Con arm, respectively) completed this trial (Fig. 1).

Patient characteristics

Division proved successful with very similar patient characteristics (Table 1). The proportion of hypertension and lipoprotein(a) level in CR arm were higher than those in Con arm ($P=0.0196$, $P=0.015$, respectively), and fibrinogen level in CR arm was lower than that in Con arm ($P=0.031$). There was no significant difference in the distribution of diseased blood vessels between the two groups (all $P>0.05$) (Table 1).

Table 1
Demographic and baseline clinical characteristics.

Variables	CR arm (N = 37)	Con arm (N = 64)	P
Age	65.86 ± 8.61	61.95 ± 10.90	0.0640
Smoking	9 (24%)	11 (17%)	0.3859
Alcohol	3 (8%)	6 (9%)	0.8830
Hypertension	19 (51%)	18 (28%)	0.0196
Diabetes	8 (21%)	9 (14%)	0.3279
Hyperlipidemia	3 (8%)	3 (4%)	0.7919
Drugs			
Statins	3 (8%)	4 (6%)	0.9583
Calcium channel blockers	6 (16%)	9 (14%)	0.7693
Antimicrobials	2 (5%)	6 (9%)	0.7419
Vessels			
Single vessel lesion	13 (35%)	20 (31%)	0.6884
Multivessel lesions	24 (64%)	44 (68%)	
Left coronary artery	19 (51%)	28 (43%)	0.5969
Right coronary artery	0 (0%)	1 (1%)	
Both	18 (48%)	35 (54%)	
Stents			0.1233
One	23 (62%)	49 (76%)	
More	14 (37%)	15 (23%)	
Cardiovascular events	4 (10%)	1 (1%)	0.0589
WBC (109/L)	6.85 ± 2.07	7.71 ± 2.45	0.075
Hb (g/L)	129.32 ± 16.56	132.83 ± 18.65	0.346
PLT (109/L)	161.05 ± 50.06	170.09 ± 45.64	0.357
CRP (mg/L)	9.59 ± 18.97	7.53 ± 13.85	0.629
TG (mmol/L)	1.96 ± 1.28	1.99 ± 1.07	0.918
TC (mmol/L)	3.98 ± 1.15	4.75 ± 6.65	0.489
HDL-C (mmol/L)	1.26 ± 0.60	1.23 ± 0.55	0.273

Variables	CR arm (N = 37)	Con arm (N = 64)	<i>P</i>
LDL-C (mmol/L)	2.13 ± 0.86	1.95 ± 0.65	0.217
Lpa (mg/L)	280.35 ± 215.06	178.23 ± 159.76	0.015
Glu (mmol/L)	6.60 ± 2.91	6.14 ± 1.83	0.329
ALT (U/L)	29.65 ± 18.92	30.72 ± 0.87	0.798
UA (umol/L)	327.35 ± 73.56	347.63 ± 93.57	0.261
BUN (mmol/L)	5.49 ± 1.62	6.10 ± 2.94	0.181
Cr (umol/L)	81.57 ± 41.38	75.52 ± 30.78	0.405
INR	1.08 ± 0.25	1.08 ± 0.11	0.988
FIB (g/L)	2.87 ± 1.19	4.40 ± 0.70	0.031

Identification of Diagnostic Signatures

Through logistic regression analysis, hypertension (OR = 3.115, 95% CI: 1.201 ~ 8.077) and low level of fibrinogen (OR = 1.919, 95% CI: 1.081 ~ 3.408) were found to be related to independent risk factors for CR. Lastly, there were no differences in tongue color, tongue form and tongue coating (all $P > 0.05$) (Table 2). However, CR arm had a higher classification in tongue color and sublingual complex compared to Con arm ($P < 0.05$, $P < 0.01$, respectively) (Table 3).

Table 2
Tongue diagnostic parameters characteristics.

Variables	CR arm	Con arm	<i>P</i>
Tongue form			
Size			0.9326
Fat	18(47%)	31(48%)	
Normal	16(43%)	29(45%)	
Lean	3(8%)	4(6%)	
Teeth marks			0.4129
Yes	12(32%)	26(41%)	
No	25(68%)	38(59%)	
Crack			0.8835
Yes	5(14%)	8(13%)	
No	32(86%)	56(88%)	
Tongue coating color			0.9274
White	26(70%)	45(70%)	
Yellow	8(22%)	15(23%)	
Dark gray	3(8%)	4(6%)	
Tongue coating proper			
			0.2264
Thick	8(22%)	8(13%)	
Thin	29(78%)	56(88%)	
			0.9127
Smooth	18(49%)	33(52%)	
Moist	3(8%)	6(9%)	
Dry	16(43%)	25(39%)	
Greasy			0.8732
Yes	11(30%)	20(31%)	
No	26(70%)	44(69%)	
Exfoliative			0.4453

Variables	CR arm	Con arm	<i>P</i>
Yes	6(16%)	7(11%)	
No	31(84%)	57(89%)	

Table 3
Tongue diagnostic parameters classification.

Variables	CR arm	Con arm	<i>P</i>
Tongue color classification			< 0.05
0	4	15	
1	5	8	
2	9	21	
3	19	20	
Sublingual complex classification			< 0.01
0	3	10	
1	4	18	
2	14	26	
3	16	10	

Development of TDP-Based Diagnostic Signatures

The ROC curve analysis was performed to compare the sensitivity and specificity of diagnostic prediction in TDP-based diagnostic signatures. The AUC value was obtained from ROC curve analysis. In this study, Model 1 (tongue color classification), Model 2 (sublingual complex classification), Model 3 (Model 1 + 2) and Model 4 (Model 3 + hypertension + fibrinogen) reached AUC values of 0.613, 0.679, 0.692 and 0.766, respectively (Fig. 2), demonstrating that the diagnostic power of this signatures was credible. In addition, the risk score was calculated according to the signatures as follow: Risk score = $1/[1 + e^{-(0.186 \times \text{tongue color classification} + 0.658 \times \text{sublingual complex classification} + 1.252 \times \text{hypertension} + 0.718 \times \text{fibrinogen} - 4.509)}]$.

Validation of TDP-Based Diagnostic Signatures

Of 20 patients, 11 cases were CR and 9 cases were non-CR. TDP-based diagnostic signatures correctly diagnosed 9 in CR and 8 in non-CR with an accuracy rate of 81.9%.

Discussion

In the last few decades, significant breakthrough has been made in our comprehension of the development and therapy of CAD[26]. As angiography continues to mature, more and more hospitals begin to implement PCI, which also benefits more patients. However, despite the excellent anti-platelet aggregation of dual antiplatelet therapy after PCI, there are still relevant studies showing that the response of platelets to clopidogrel remains highly diverse among individuals[27, 28], and some patients may still suffer from in-stent thrombosis, recurrent myocardial infarction and other ischemic events during the treatment of clopidogrel[23, 29]. Hence, it is necessary to determine the biological characteristics regarding CR.

There are several commonly used risk scores for CAD. The GRACE risk score has been developed into an application and it has been integrated into electronic medical records systems used in daily clinical management of ACS patients worldwide. The United Kingdom National Institute for Health and Clinical Excellence guideline has recommended employing the GRACE risk score since 2010[30]. The purpose of CRUSADE Bleeding Score is to help clinicians estimate a patient's baseline risk of in-hospital major bleeding during non-ST-segment elevation myocardial infarction (NSTEMI)[31]. The SYNTAX score is a unique tool to score complexity of CAD[32]. The NERS score II, similar to the conventional NERS score, is more predictive of major adverse cardiac events than the SYNTAX score in unprotected left main coronary artery patients after implantation of a drug-eluting stent[33]. Later, apolipoprotein A1 was identified associated with SYNTAX score in patients with a NSTEMI and apolipoprotein A1 < 1.07 g/L may have more complex coronary artery lesions[34]. Recently studies found that lipids[35], plasma B-type natriuretic peptide level before PCI[36] and circulating HtrA2[37] showed promise as a novel potential biomarker for identify ischemia-reperfusion injury after STEMI. The CAMI-NSTEMI[38, 39] and SCAMI-NSTEMI[40] score can serve as a useful tool facilitating rapid risk assessment among a broader spectrum of patients admitted owing to NSTEMI. However, there are no relevant risk scores that can be used to diagnose CR in CAD patients after PCI. We innovatively developed and validated a TDP-based diagnostic signatures in CAD patients with CR after PCI that is not described in the current guidelines. When we combined tongue color classification and sublingual complex classification (Model 3), the AUC value is 0.692. While hypertension and fibrinogen were added into diagnostic signatures, the AUC reached 0.766, demonstrating that the diagnostic power of this signatures was credible.

Furthermore, we developed and validated a TDP-based diagnostic signatures that is significantly associated with CR prediction, making it a favorable and practical stratified for risk classification in CR. In search of an optimal signature with diagnostic prediction, it could be proven that the diagnostic power of the TDP-based diagnostic signatures is accepted. This useful strategy for the vigorous selection of markers has vast application potential in other diseases. The high diagnostic categorization performance of the TDP-based diagnostic signatures is assuredly due to our idiographic reanalysis strategy. To identify reliable diagnostic signatures of CR, we utilized methods that are specifically designed to perform resolutely. As such, our TDP-based diagnostic signatures model can serve as personalized, single-sample estimate of survival in NBL patients and may be promptly incorporated into clinical utility.

Conclusions

Herein, we performed and proposed the first TDP-based diagnostic signatures analysis in CAD patients with CR after PCI. However, there are not no limitations to this study, although the TDP-based diagnostic signatures is robust. We only included population undergone PCI, so other high-quality clinical evidence is needed before the model is applied to all CAD patients as a clinically useful tool. Moreover, this model included 101 patients from a large tertiary referral center, however, the clinical sample size is relatively small. Therefore, further larger, multicenter prospective studies are desired to validate these findings.

Abbreviations

AUC: area under the receiver operating characteristic curve, CAD: coronary artery disease, CR: clopidogrel resistance, NSTEMI: non-ST-segment elevation myocardial infarction, PCI: percutaneous coronary intervention, ROC: receiver operating characteristic, TCM: traditional Chinese medicine, TDP: tongue diagnostic parameters.

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee at The Affiliated Jiangning Hospital of Nanjing Medical University (No. 20150221), and patients provided signed written informed consent before enrollment and were able to withdraw from the study at any time and no explanation was required.

Consent for publication

Not applicable.

Availability of data and materials

All data generated or analysed during this study are included in this published article.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BL and NG conceived, designed, or planned the study. BL, CHL, and JL acquired the data. BL, CHL, and NG analyzed the data. All authors helped interpret the results. CHL and NG provided study materials or patients. BL drafted the manuscript. All authors revised and reviewed this work, and all authors gave their final approval of the submitted manuscript.

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Figures

Figure 1

Patient flow diagram.

Figure 2

ROC curves for diagnostic signatures.