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EDITORIAL

Reply to "The Predictive Role of Glycocalyx Assessment in Subjects with Cardiovascular Risk Factors Within and Beyond SCORE"

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We appreciate the constructive comments by Dr. Kawada on our study [1]. In our study, we have constructed different multivariate models to address the predictive role of PBR for cardiovascular events among participants without established cardiovascular disease. In all models, PBR was found to be an independent and additive predictor to traditional risk factors [2].

In multivariate analysis, we have built four different models, each one including a different number of variables ranging from 2 to 10 variables after the adjustment for PBR, which indeed exceeds the number of variables that the rule of one in ten suggests that should be included in a multivariable model. The main reason to avoid too many variables is the risk of overfitting which could lead to unambiguous results. The rule of one in ten has been introduced and is being used in many cases to define how many predictors should be estimated to avoid harming generalizability. However, this rule has also been criticized for being too conservative as a general recommendation, and there are also studies suggesting that the ratio of events per predictive variable is not a reliable statistic for selecting the number of variables for a multivariable model [3]. In fact, there is no limit in the number of variables that can be analyzed in a multivariable model as long as the predictive validation of the obtained model is consistent.

In our analysis, the predictors had been predetermined based on traditional risk factors for cardiovascular disease and/or medication known to affect prognosis, in order to address the literature as well as our hypothesis. Additionally, the hazard ratios obtained were of the same direction and significance in all our models before and after the adjustment for PBR, showing that our results are consistent and robust and the statistically significant variables were not affected by the number of variables included in the model. Finally, the most important hypothesis that endothelial glycocalyx has an independent and additive prognostic value to traditional risk factors in primary prevention population was explored in a model including SCORE (which includes smoking, blood pressure, cholesterol levels, sex, and age) and parameters with prognostic value not included in SCORE such as diabetes, family history of CAD, ACEi/ARB, and lipid lowering medication (only 5 covariates for 57 adverse events and thus complying to the rule of 1 covariate for every 10 cases) as well as in model including only SCORE (only 2 covariates), excluding any likelihood of overfitting in the multivariable analysis.

The intra- and inter-observer variances of PBR measurement are 4.3% and 5.2%, respectively [2, 4]. Thus, PBR evaluation is considered a reliable and reproducible method to assess thickness of glycocalyx layer. On the contrary, the inter-assay and intra- assay variances of sydencan-1, (a blood biomarker used to assess endothelial glycocalyx integrity) quantification by ELISA are 10.2% and 6.2%, respectively [5, 6]. Similarly, the inter-assay and intra-assay variances of the commercially available kits used for quantification of serum heparan sulfate exceed 5.7 and 6.4% [7]. Thus, syndencan-1 and other blood biomarkers seem to be a less precise metric of endothelial glycocalyx integrity, compared to RBR evaluation by microscopy of the sublingual microvessel [5]. Finally, measurement of N-terminal pro-B-type natriuretic peptide is not recommended as a screening tool for adverse events in primary prevention population in the current ESC guidelines [8] and thus was not included in our analysis. However, it would be interesting to explore the additive prognostic value of PBR to NT-proBNP regarding progression to heart failure particularly in high risk cohorts such as the diabetic subjects.

Editor-in-Chief Enrique Lara-Pezzi oversaw the review of this article.

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Table 1 Cox proportional hazard models after addition of PBR5-25 to SCORE

Subgroup	*N* (%)	*HR* (95% *CI*)	*p*-value	*p*-value of chi-square change
DM	72 (12%)	6.28 (1.05–40.38)	.043	.045
Hypertension	168 (28%)	5.91 (1.04–34.51)	.041	.042
Current smoking	261 (43.5%)	7.47 (1.47–39.45)	.018	.014
Hyperlipidemia	179 (29.8%)	9.36 (1.23–41.22)	.028	.030

The table shows the hazard ratios of PBR5-25 with their respective *p*-values after addition of PBR5-25 to SCORE and the respective *p*-value of chi-square change for each subgroup of our initial sample

HR hazard ratio, 95% *CI* 95% confidence intervals, *DM* diabetes mellitus

In a further analysis not included in our previous paper [2], we have also observed that PBR serves as a significant independent and additive predictor to SCORE regarding cardiovascular events in each one of the traditional risk factors and diabetes (Table 1). More specifically, PBR5-25 was a statistically significant predictor for (MACEs), when added to SCORE (a) in diabetics (*p* for chi-square change: 0.045), (b) hypertensives (*p* for chi-square change: 0.042), (c) current smokers (*p* for chi-square change: 0.014), and (d) patients with hyperlipidemia (*p* for chi-square change: 0.030).

Thus, assessment of endothelial glycocalyx by PBR5-25 confers significantly and additively to prediction of MACEs in the presence of each one of the traditional risk factors as well as in high risk group of patients such as the diabetic despite risk stratification using the validated SCORE.

Data Availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of Interest The authors declare no competing interests.

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