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Short communication

Enhanced NOX-2 derived oxidative stress in offspring of patients with early myocardial infarction

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ABSTRACT

Background: Offspring of patients with early myocardial infarction have a higher risk to develop cardiovascular events; the underlying physiopathology is still unclear. Several lines of evidence support a role for oxidative stress in atherogenesis and NADPH oxidase-2 (NOX-2) is considered a major source of $O2^-$ in human. Furthermore, oxidative stress regulates arachidonic acid metabolism via activation of platelet phospholipase-A2.
The aim of this study was to address NOX-2 activity as well as serum thromboxane B2 (TXB2) and 8-isoPGF2-alpha in offspring of patients with premature myocardial infarction.
Methods: Ninety-two consecutive subjects, including 46 offspring of patients with premature myocardial infarction and 46 healthy subjects (HS) matched for age and gender, were recruited.
A cross sectional study was performed to compare serum activity of soluble NOX-2-dp (sNOX-2-dp), blood levels of isoprostanes and serum TXB2 in these two groups.
Results: Compared with HS, offspring of patients with early myocardial infarction had higher values of serum TxB2, isoprostanes and sNOX-2-dp. Bivariate analysis in the overall population showed that serum sNOX-2-dp levels were significantly associated with serum isoprostanes and TXB2. A multiple linear regression analysis was performed to define the independent predictors of sNOX-2-dp. Serum isoprostanes (SE: 0.07; standardized coefficient β: 0.579; $P < 0.001$) and TXB2 levels (SE: 0.06; standardized coefficient β: 0.211; $P < 0.001$) were significantly associated to sNOX-2-dp (R2: 0.42).
Conclusion: This study shows that Nox-2 activation is a key determinant of oxidative stress and platelet activation in offspring of patients with premature myocardial infarction.

1. Introduction

Coronary heart disease (CHD) is the most prevalent cause of vascular death in the world [1]. In addition to atherosclerotic risk factors such as dyslipidemia, diabetes or hypertension, CHD family history is also an independent risk factor for subsequent development of atherosclerotic complications [2]. Even if atherosclerotic risk factors often account for the family history of CHD, such relationship is not fully established [3]. As shown by previous meta-analyses, when the parent's myocardial infarction occurred before the age of 50 years, a greater cardiovascular risk was observed in offspring [4].

Oxidative stress plays a key role in the process of atherosclerosis [5]. Several ROS-generating enzymes, including myeloperoxidase, xanthine oxidase and NADPH oxidase may be implicated in the atherogenesis [6]. Experimental studies performed in animal models suggest vasoconstrictive and aggregating properties for NADPH oxidase-2 (NOX-2) [6]. Furthermore, in patients with genetic deficiency of NOX-2, an enhanced flow-mediated dilation along with a reduced atherosclerotic burden in carotid artery has been documented [7–9]. NOX-2 plays also an important role in the thrombotic process as documented in patients with hereditary deficiency of NOX-2, who display impaired platelet recruitment

Abbreviations: NADPH oxidase, Nicotinamide-adenine dinucleotide phosphate oxidase; 8-isoPGF2α, 8-isoprostaglandin F2α; BMI, body mass index; CHD, coronary heart disease.

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and thrombus growth. NOX-2 also plays a role in animals knock-out for the enzymes showing reduced platelet-dependent thrombus formation [10].

While the relationship between platelet function and premature atherosclerosis has been previously reported showing platelet over-activation in offspring of patients who experienced myocardial infarction before 50 years of age [11], no data so far exist regarding the interplay between NOX-2-related oxidative stress and premature cardiac complications. However, the underlying mechanism was not clarified [11]. We have discovered that platelets express NOX-2, which plays a role in favoring formation of platelet eicosanoids implicated in aggregation, i.e. the isoprostane 8-isoPGF2alpha [10]. Furthermore, clinical studies documented a significant association between urinary excretion of isoprostane and cardiovascular disease reinforcing the concept that NOX-2-derived oxidative stress may be implicated in atherosclerotic burden [12]. Until now, no data exist on the behavior of NOX-2 and 8-isoPGF2alpha in offspring of patients with premature CHD. We speculated that in these subjects NOX-2 is over-activated and is associated with 8-isoPGF2alpha over-production, which may contribute to premature coronary thrombosis. Furthermore, based on the fact that oxidative stress regulates arachidonic acid metabolism via stimulation of phospholipase-A2, we also investigated if NOX-2 activity and thromboxane B2 (TXB2) production were associated. To address these issues, NOX-2 activity as well as serum TxB2, which maximally reflects platelet thromboxane A2 biosynthesis [13], and 8-isoPGF2alpha have been measured in offspring of patients with premature CHD and in children matched for sex, age and atherosclerotic risk factors without a family history of premature CHD.

2. Materials and methods

Ninety-two consecutive subjects, including 46 patient offspring of with premature myocardial infarction and 46 healthy subjects (HS) matched for age and gender, were enrolled. Further details for materials and methods and statistical analysis are reported in the supplementary data.

3. Results

Clinical characteristics of the population are reported in supplemental data (See Table 1 in supplemental data). No significant difference was found between the groups for BMI, cholesterol levels, triglycerides, systolic and diastolic blood pressure and fasting blood glucose (See Table 1 in supplemental data). Premature myocardial infarction occurred at 42.7 ± 5.7 years old in 41 males (42.4 ± 5.8 years old) and 5 females (mean age 45.0 ± 4.5 years old); fatal myocardial infarction occurred in 4 males (mean age: 37 years old) and 2 females (mean age: 42 years old).

Compared with HS, offspring of patients with early myocardial infarction had higher values of serum TXB2 (Fig. 1 panel A), isoprostanes (Fig. 1 panel B) and sNOX-2-dp (Fig. 1 panel C). Conversely, no significant difference in levels for C-Reactive Protein was found (0.5 ± 0.21 vs 0.42 ± 0.30 mg/L, $p = 0.123$) (Supplemental data, Table 1).

Bivariate analysis in the overall population showed that serum sNOX-2-dp levels were significantly associated with serum isoprostanes ($Rs = 0.530$; $p < 0.001$) and with serum TxB2 ($Rs = 0.251$; $p = 0.01$); furthermore, serum isoprostanes were associated with TXB2 serum levels ($Rs = 0.243$; $p = 0.020$).

A multiple linear regression analysis, including the variables linearly associated with the dependent variable, was performed to define the independent predictors of sNOX-2-dp. Serum isoprostanes (SE: 0.07; standardized coefficient β: 0.579; $p < 0.001$) and serum TXB2 levels (SE: 0.06; standardized coefficient β: 0.211; $p < 0.001$) were significantly associated to sNOX-2-dp (R2: 0.42).

Furthermore, to evaluate if the high levels of serum TXB2, isoprostanes and sNOX-2-dp were related to the coexistence of traditional cardiovascular risk factors or to the family history of CHD per se, we divided the population composed of offspring of patients with early myocardial infarction in two groups: children with or without cardiovascular risk factors. This analysis showed no differences between

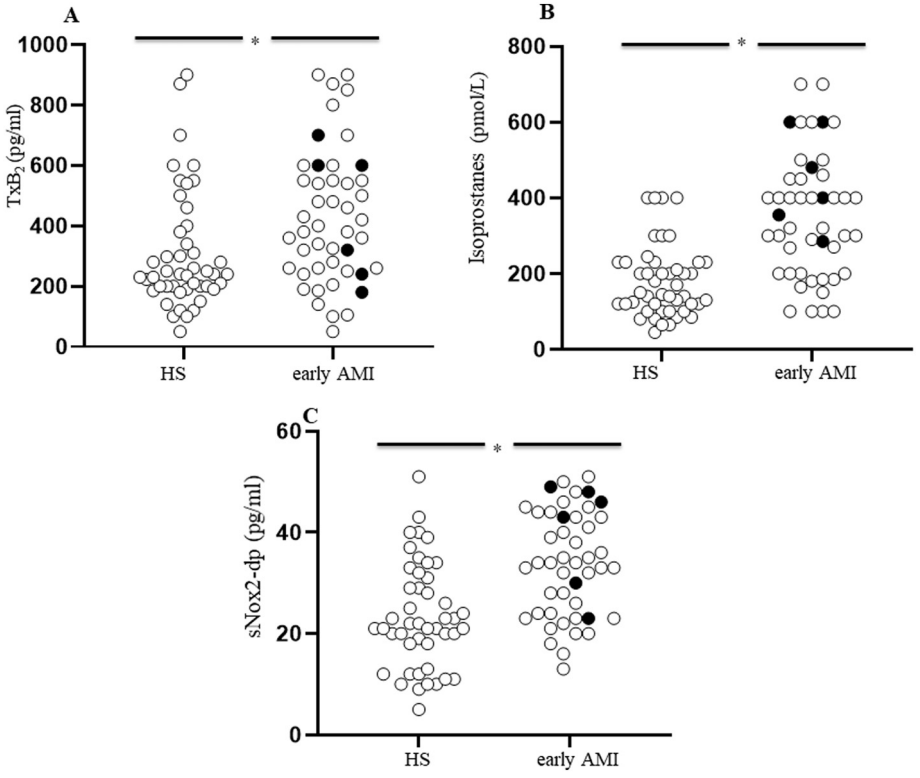

Fig. 1. Serum TXB2, isoprostanes, NOX2 levels in offspring of patients with early acute myocardial infarction (AMI) and healthy subjects (HS) (white dots); black dots represent siblings of patients with fatal acute myocardial infarction.

the two groups for the above cited variables (see Fig. 2 and supplemental data, Table 2).

It is of interest to note that, offspring of patients who had fatal myocardial infarction showed significant higher levels of sNOX-2-dp and serum isoprostanes compared to those with non-fatal myocardial infarction (respectively 42 ± 7 vs 32 ± 10 pg/mL, p = 0.01 and 472 ± 106 vs 334 ± 163 pmol/L, p = 0.02).

4. Discussion

This study provides the first report attesting that offspring of patients with premature myocardial infarction have high NOX-2 activation, serum isoprostanes and TXB2 levels, and suggests a potential role for oxidative stress in platelet activation in this population at high cardiovascular risk.

NOX-2 is considered a key target in atherosclerosis [14,15] and platelet activation [6]. Reactive oxygen species (ROS) generated by NOX-2 inactivate nitric oxide, an endogenous platelet inhibitor [10]. On the other hand, ROS generated by NOX-2 may interact with arachidonic acid to produce isoprostanes, which are molecules contributing to spread platelet aggregation [16]. Consistent with this, previous studies showed that NOX-2 down-regulation is associated with impaired isoprostanes formation and enhanced NO generation [7,17].

In this study we demonstrated, compared to controls, higher serum levels of sNOX-2-dp and isoprostanes levels in offspring of patients with premature myocardial infarction. This increased oxidative stress, observed in this high risk cardiovascular population, is in accordance with a previous paper by Kelishadi that found high OX-LDL levels in children with a positive family history of premature CHD [18].

Previous studies showed that NOX-2 generated oxidative stress contributes to isoprostanes formation and to platelet activation [6]. The increased levels of TXB2 observed in our study confirm, as previously showed by Lanza et al. [11], that children of patients with precocious myocardial infarction have increased platelet activation. A potential implication of these findings is that down-regulating NOX-2 might be useful to modulate platelet activation and eventually cardiovascular risk but prospective studies (e.g. with statins or antioxidants) are necessary to establish a cause-effect relationship between NOX-2-related oxidative stress and cardiovascular events.

Previous studies showed a higher incidence of classic cardiovascular risk factors as diabetes, insulin resistance, metabolic syndrome and dyslipidemia in the offspring of patients with premature CHD [19,20]. In our study, a subgroup analysis of the offspring of patients with early myocardial infarction, selected according to the presence of cardiovascular classic risk factors, did not find significant differences for the levels of serum TXB2, isoprostanes and sNOX-2-dp. Further studies with larger sample size need to address this issue.

Another interesting finding, even if limited by the small sample size, is that offspring of patients who had fatal myocardial infarction showed significant higher levels of sNOX-2-dp and serum isoprostanes compared to those with non-fatal myocardial infarction. Future studies will have to demonstrate if this particular population must be considered at higher cardiovascular risk.

Regarding CRP values, we did not find differences between offspring of patients with premature myocardial infarction and controls. This finding is in accordance with previously reported data by Lanza et al. [11] and, apparently, showed no significant role for sub-clinical inflammation to increase NOX-2 activation in this population compared to controls.

The study had limitations. The mechanism accounting for platelet activation is not fully clarified by the present study; we cannot exclude in offspring of patients with premature myocardial infarction that polymorphism of NADPH oxidase, e.g. in the p22-PHOX (CYBA) gene or other genes, could increase NOX-2 and platelet activation.

Furthermore, we did not evaluate other NADPH isoforms, such as NOX1 and NOX4 that could also contribute to increase oxidative stress.

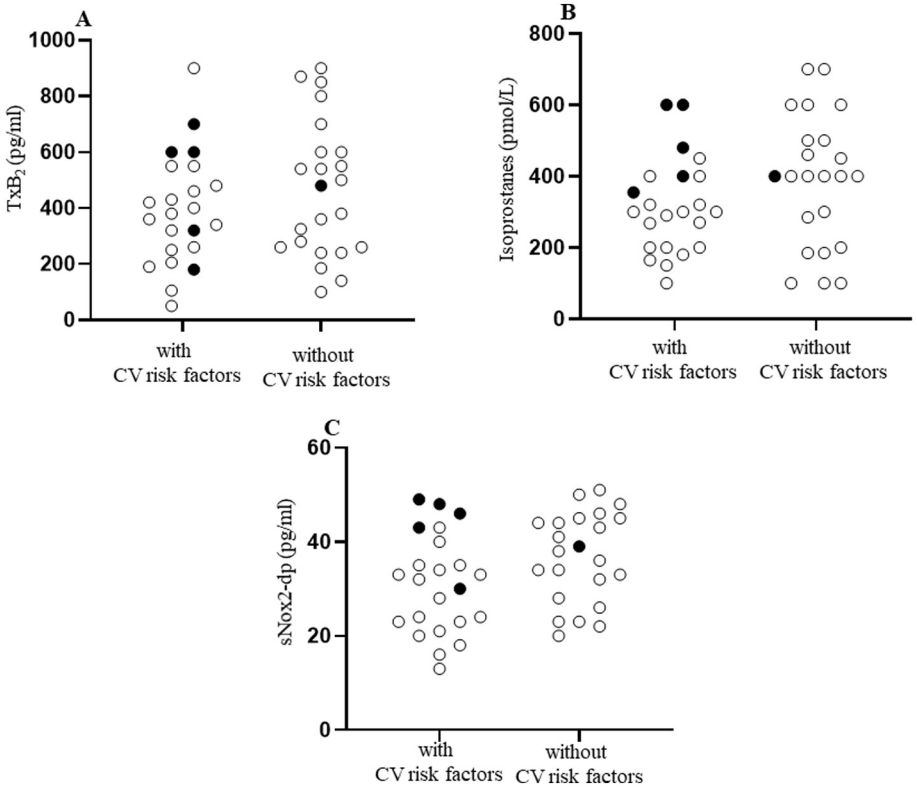

Fig. 2. Serum TXB2, isoprostanes, NOX2 levels in offspring of patients with early acute myocardial infarction with and without cardiovascular risk factors; black dots represent siblings of patients with fatal acute myocardial infarction.

In conclusion, our study shows that NOX-2 activation is a key determinant of oxidative stress and platelet activation in offspring of patients with premature myocardial infarction.

Financial disclosure

All the authors have no financial relationships relevant to this article to disclose.

Conflict of interest

The authors report no relationships that could be construed as a conflict of interest.

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None.

Author contributions

Prof Loffredo and Martino F. conceptualized and designed the study, drafted the initial manuscript, reviewed and revised the manuscript. Dr. Battaglia, Dr. Martino E., and Professors Zicari and De Castro collected data, carried out the initial analyses, and reviewed and revised the manuscript. Dr. Carnevale, Cammisotto and Peruzzi performed laboratory analyses and reviewed and revised the manuscript. Prof. Violi, Barillà and Duse conceptualized and designed the study, coordinated and supervised data collection, and critically reviewed the manuscript for important intellectual content.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2019.05.014.

References

[1] W. Herrington, B. Lacey, P. Sherliker, J. Armitage, S. Lewington, Epidemiology of atherosclerosis and the potential to reduce the global burden of Atherothrombotic disease, Circ. Res. 118 (2016) 535–546.
[2] R.H. Myers, D.K. Kiely, L.A. Cupples, W.B. Kannel, Parental history is an independent risk factor for coronary artery disease: the Framingham study, Am. Heart J. 120 (1990) 963–969.
[3] M.B. Andresdottir, G. Sigurdsson, H. Sigvaldason, V. Gudnason, Fifteen percent of myocardial infarctions and coronary revascularizations explained by family history unrelated to conventional risk factors. The Reykjavik cohort study, Eur. Heart J. 23 (2002) 1655–1663.
[4] M. Weijmans, Y. van der Graaf, J.B. Reitsma, F.L. Visseren, Paternal or maternal history of cardiovascular disease and the risk of cardiovascular disease in offspring. A systematic review and meta-analysis, Int. J. Cardiol. 179 (2015) 409–416.
[5] R. Stocker, J.F. Keaney Jr., Role of oxidative modifications in atherosclerosis, Physiol. Rev. 84 (2004) 1381–1478.
[6] F. Violi, R. Carnevale, L. Loffredo, P. Pignatelli, J.I. Gallin, NADPH Oxidase-2 and Atherothrombosis: insight from chronic granulomatous disease, Arterioscler. Thromb. Vasc. Biol. 37 (2017) 218–225.
[7] F. Violi, V. Sanguigni, R. Carnevale, A. Plebani, P. Rossi, A. Finocchi, et al., Hereditary deficiency of gp91(phox) is associated with enhanced arterial dilatation: results of a multicenter study, Circulation. 120 (2009) 1616–1622.
[8] L. Loffredo, R. Carnevale, V. Sanguigni, A. Plebani, P. Rossi, C. Pignata, et al., Does NADPH oxidase deficiency cause artery dilatation in humans? Antioxid. Redox Signal. 18 (2013) 1491–1496.
[9] F. Violi, V. Sanguigni, L. Loffredo, R. Carnevale, B. Buchetti, A. Finocchi, et al., Nox2 is determinant for ischemia-induced oxidative stress and arterial vasodilatation: a pilot study in patients with hereditary Nox2 deficiency, Arterioscler. Thromb. Vasc. Biol. 26 (2006) e131–e132.
[10] P. Pignatelli, R. Carnevale, S. Di Santo, S. Bartimoccia, V. Sanguigni, L. Lenti, et al., Inherited human gp91phox deficiency is associated with impaired isoprostane formation and platelet dysfunction, Arterioscler. Thromb. Vasc. Biol. 31 (2011) 423–434.
[11] G.A. Lanza, G. Scalone, L. Barone, F. Infusino, I. Coviello, A. Di Monaco, et al., Platelet reactivity and endothelial function in children of patients with early acute myocardial infarction, Eur. Heart J. 32 (2011) 2042–2049.
[12] L. Loffredo, R. Carnevale, R. Cangemi, F. Angelico, T. Augelletti, S. Di Santo, et al., NOX2 up-regulation is associated with artery dysfunction in patients with peripheral artery disease, Int. J. Cardiol. 165 (2013) 184–192.
[13] I.A. Reilly, G.A. FitzGerald, Inhibition of thromboxane formation in vivo and ex vivo: implications for therapy with platelet inhibitory drugs, Blood. 69 (1987) 180–186.
[14] L. Loffredo, A.M. Zicari, F. Occasi, L. Perri, R. Carnevale, F. Angelico, et al., Endothelial dysfunction and oxidative stress in children with sleep disordered breathing: role of NADPH oxidase, Atherosclerosis. 240 (2015) 222–227.
[15] L. Loffredo, A.M. Zicari, F. Occasi, L. Perri, R. Carnevale, F. Angelico, et al., Role of NADPH oxidase-2 and oxidative stress in children exposed to passive smoking, Thorax. 73 (2018) 986–988.
[16] R. Carnevale, L. Loffredo, V. Sanguigni, A. Plebani, P. Rossi, C. Pignata, et al., Different degrees of NADPH oxidase 2 regulation and in vivo platelet activation: lesson from chronic granulomatous disease, J. Am. Heart Assoc. 3 (2014), e000920.
[17] F. Violi, P. Pignatelli, C. Pignata, A. Plebani, P. Rossi, V. Sanguigni, et al., Reduced atherosclerotic burden in subjects with genetically determined low oxidative stress, Arterioscler. Thromb. Vasc. Biol. 33 (2013) 406–412.
[18] R. Kelishadi, M. Sabri, N. Motamedi, M.A. Ramezani, Factor analysis of markers of inflammation and oxidation and echocardiographic findings in children with a positive family history of premature coronary heart disease, Pediatr. Cardiol. 30 (2009) 477–481.
[19] M. Magnusson, P. Burri, O. Melander, A clinically confirmed family history for early myocardial infarction is associated with increased risk of obesity, insulin resistance and metabolic syndrome, J. Hypertens. 30 (2012) 948–953.
[20] L.S. Rallidis, N.H. Papageorgakis, A.A. Megalou, N.J. Exadactylos, G.K. Tsitouris, E.G. Papasteriadis, High incidence of dyslipidaemia in the offspring of Greek men with premature coronary artery disease, Eur. Heart J. 19 (1998) 395–401.