International Journal of Cardiology 276 (2019) 289–292

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard

Short communication

# Correlation between $CD4^+$ $CD28^{null}$ T lymphocytes, regulatory T cells and plaque rupture: An Optical Coherence Tomography study in Acute Coronary Syndromes☆

Aureliano Ruggio [1], Daniela Pedicino [1], Davide Flego, Rocco Vergallo, Anna Severino, Claudia Lucci, Giampaolo Niccoli, Carlo Trani, Francesco Burzotta, Cristina Aurigemma, Antonio Maria Leone, Antonino Buffon, Alessia D'Aiello, Luigi Marzio Biasucci, Filippo Crea [2], Giovanna Liuzzo *,[2]

*Department of Cardiovascular and Thoracic Sciences, Catholic University of the Sacred Heart, Rome, Italy*
*Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy*

ARTICLE INFO

*Article history:*
Received 5 June 2018
Received in revised form 25 July 2018
Accepted 31 August 2018
Available online 7 September 2018

*Keywords:*
Acute Coronary Syndromes
Plaque rupture
Inflammation
Optical Coherence Tomography
Precision medicine

ABSTRACT

*Background:* A sizeable proportion of patients with Acute Coronary Syndromes (ACS) shows a unique adaptive immune system profile, associated to a worse outcome, characterized by higher $CD4^+$ $CD28^{null}$ T-cells, lower regulatory T-cells (Treg) and increased $CD4^+$ $CD28^{null}$ /Treg ratio. We sought to investigate the correlation between $CD4^+$ $CD28^{null}$ T-cells, Treg, $CD4^+$ $CD28^{null}$ /Treg ratio and plaque phenotype as assessed by Optical Coherence Tomography (OCT).

*Methods:* Peripheral blood mononuclear cells (PBMC) were collected from 30 Non-ST Elevation Myocardial Infarction (NSTEMI) patients, sub-grouped according to OCT analysis of culprit lesions into two cohorts: Ruptured Fibrous Cap (NSTEMI-RFC, n = 12) and Intact Fibrous Cap (NSTEMI-IFC, n = 18). Stable Angina patients (SA, n = 18) were used as controls. We examined the frequency of $CD4^+$ $CD28^{null}$ and Treg (defined as $CD4^{+-}$ $CD25^{high}$ $CD127^{low}$ $Foxp3^+$ T-cells) by flow-cytometry.

*Results:* $CD4^+$ $CD28^{null}$ frequency (median, range) was significantly higher in NSTEMI-RFC patients (17.3%, 12.5–33.8) as compared with NSTEMI-IFC (3.8%, 0.3–14.1) and SA (3%, 0.6–17.7) ($P < 0.001$ for all comparisons). We also found a higher $CD4^+$ $CD28^{null}$ /Treg ratio in NSTEMI-RFC patients (6.6%, 3.7–13.9) than in NSTEMI-IFC (1.6%, 0.3–5.2) and SA (1.2%, 0.3–8.7) ($P < 0.001$ for all comparisons). Finally, there was an inverse correlation between $CD4^+$ $CD28^{null}$ /Treg ratio and cap-thickness ($R = -0.44$; $P = 0.002$).

*Conclusion:* Patients with NSTEMI presenting with RFC as culprit lesion at OCT evaluation have a specific perturbation of adaptive immunity, mostly involving $CD4^+$ $CD28^{null}$ T- cells and Tregs, as compared with patients with IFC and SA. This specific imbalance of T-cells might play a key role in fibrous cap thinning, predisposing atherosclerotic plaque to rupture.



## 1. Introduction

A perturbation of adaptive immunity, mostly involving $CD4^+$ T-cells repertoire, has been observed in patients with Acute Coronary Syndromes (ACS) and systemic inflammation [1,2]. Patients with ACS have skewed T-cell differentiation, oriented toward aggressive helper T (Th) lymphocytes and defective regulatory T-cells (Treg) [2,3]. $CD4^{+-}$ $CD28^{null}$ T-cells represent an unusual subset of cytotoxic long-living lymphocytes producing Interferon-γ (IFN-γ), with increased resistance to apoptosis [4,5]. Monoclonal expansion of this subpopulation has been documented in unstable ruptured plaques [6] and it is strongly associated with recurrence of ACS [7], particularly among diabetic patients [8]. Indeed, about one third of ACS patients shows a specific imbalance of T cells, characterized by higher $CD4^+$ $CD28^{null}$ T-cells, lower Treg and increased $CD4^+$ $CD28^{null}$ /Treg ratio. This specific immune–imbalance is associated to a worse outcome, thus representing a potential useful biomarker per se [9].

Optical Coherence Tomography (OCT) is a high resolution intracoronary light-based imaging technique able to identify the plaque phenotype underlying coronary thrombosis [10,11].

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

* Corresponding author at: Department of Cardiovascular and Thoracic Sciences, Catholic University of the Sacred Heart, Fondazione Policlinico Universitario A. Gemelli IRCCS, Largo A. Gemelli, 8, 00168 Rome, Italy.
*E-mail address:* giovanna.liuzzo@policlinicogemelli.it (G. Liuzzo).

[1] Drs. Aureliano Ruggio and Daniela Pedicino have contributed equally as first authors.
[2] Drs. Filippo Crea and Giovanna Liuzzo have contributed equally as senior authors.

https://doi.org/10.1016/j.ijcard.2018.08.101
0167-5273/

Aim of the present study was to investigate the correlation between a specific immunological T-cell subsets imbalance (i.e. $CD4^+$ $CD28^{null}$ and Treg) and OCT plaque phenotype.

## 2. Methods

Complete methods are reported in Online Appendix.

### 2.1. Study population

We prospectively enrolled 30 consecutive patients admitted to our Coronary Care Unit with a diagnosis of Non-ST Elevation Myocardial Infarction (NSTEMI), undergoing OCT evaluation of the culprit coronary plaque before stent implantation for clinical reasons and with a clearly identifiable feature of the culprit plaques.

We also enrolled 18 patients with symptoms of Stable Angina (SA) lasting >12 months, angiographically confirmed coronary artery disease, no previous acute coronary events, and no overt ischemic episodes during the previous 48 h.

Exclusion criteria were presented in Online Appendix.

All individuals gave their written informed consent. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The Ethics Committee of the *Fondazione Policlinico Universitario "A. Gemelli" – Catholic University of Rome* approved the study. The authors have certified that they comply with the Principles of Ethical Publishing in the International Journal of Cardiology [12].

### 2.2. Blood sampling, PBMC isolation and flow-cytometry

Venous blood samples were taken at patient enrollment, within 24 h from symptoms onset, before undergoing coronary angiography and OCT evaluation, that were performed within 48 h in all patients (9.8 ± 3.6 h). White blood cell counts and T-cell subset distribution were analyzed on fresh blood samples. Serum cardiac troponin I (cTnI) and high-sensitivity C-reactive protein (hs-CRP) were determined at the time of hospital admission as routine measurement (see Online Appendix).

T-cell subset frequency ($CD4^+$ $CD28^{null}$ T-cells and Treg defined as $CD4^+$ $CD25^{high}$ $CD127^{low}$ $Foxp3^+$ T-cells) was examined by flow-cytometry, as previously described [3,7] and as shown in Online Appendix and Supplementary Fig. 1.

### 2.3. OCT analysis

A frequency domain OCT system (ILUMIEN OPTIS, St. Paul, MN) was used to perform OCT analysis of the culprit lesion before stent implantation. Culprit lesion was identified by angiography, electrocardiographic alterations, and/or regional wall motion abnormalities on echocardiographic assessment. NSTEMI patients were sub-grouped according to culprit lesion morphology into two cohorts: Ruptured Fibrous Cap (RFC) and Intact Fibrous Cap (IFC).

RFC was defined as the presence of fibrous cap discontinuity leading to a communication between the inner (necrotic) core and the lumen (Fig. 1, Panels A–E). Plaque rupture included also fibrous cap disruption detected over a calcified plaque according to recent proposed criteria [13–16].

IFC included both definite (the presence of an attached thrombus overlying an intact plaque) and probable erosions, defined as luminal irregularity without thrombus or thrombus without a superficial lipid or calcified plaque in the proximity of the thrombus (Fig. 1, Panels A′–E′) [14]. Finally, IFC included also smooth plaques without evidence of rupture or thrombus as recently suggested [14,15]. Patients with other culprit OCT imaged aspects such as haematoma and dissections were excluded from the study. Additional OCT features are reported in Online Appendix.

### 2.4. Statistical methods

Statistical analysis was performed with SPSS software v22.0 (IBM Corporation, Armonk, New York). Complete methods are reported in Online Appendix.

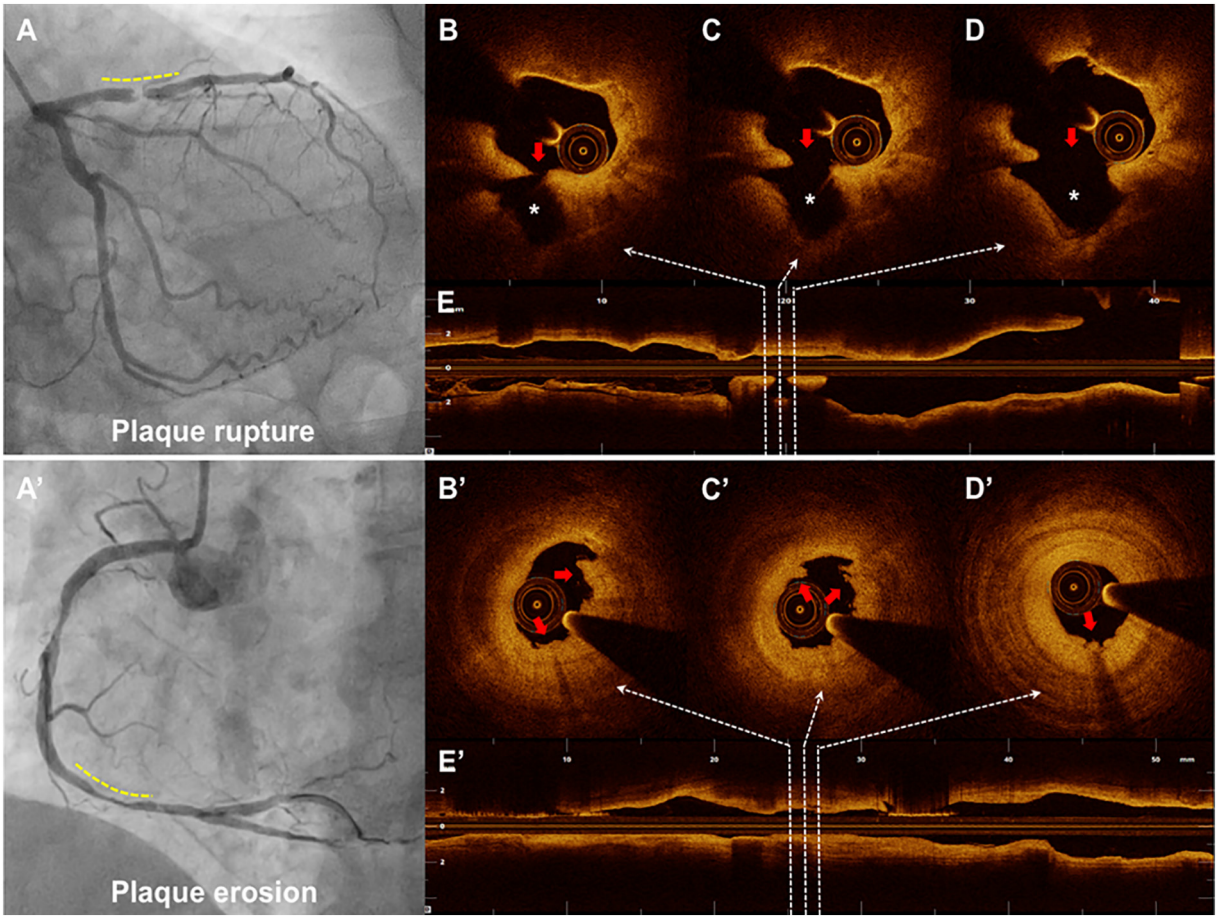


**Fig. 1.** Representative OCT images of coronary plaque rupture and plaque erosion. Coronary angiogram showing a complex, scalloped lesion (dashed yellow line) of the proximal left anterior descending artery (A). OCT imaging disclosed a coronary plaque rupture (B–D), appearing as a discontinuity of the fibrous cap (red arrows) with an emptied cavity (asterisks). A longitudinal OCT view of the culprit vessel is provided in E. Coronary angiogram showing two eccentric lesions of the right coronary artery (A′). The distal lesion (dashed yellow line) appeared to be a coronary plaque erosion at OCT imaging (B′–D′) with a discrete amount of white thrombus (red arrows) overlying an intact fibrous cap. A longitudinal OCT view of the culprit vessel is provided in E′. Abbreviations. OCT = Optical Coherence Tomography.

## 3. Results

Characteristics of study population and plaque features are reported in Supplementary Table 1.

$CD4^+$ $CD28^{null}$ frequency (median, range) was significantly higher in NSTEMI-RFC patients (17.3%, 12.5–33.8) as compared with NSTEMI-IFC (3.8%, 0.3–14.1) and SA (3%, 0.6–17.7) ($P < 0.001$ for all comparisons) (Fig. 2A). No differences were observed in Treg frequency among groups (Fig. 2B). Nevertheless, we found a higher $CD4^+$ $CD28^{null}$/Treg ratio in NSTEMI-RFC patients (6.6%, 3.7–13.9) than in NSTEMI-IFC (1.6%, 0.3–5.2) and SA (1.2, 0.3–8.7) ($P < 0.001$ for all comparisons), suggesting that an immune imbalance toward an aggressive Th-lymphocyte response might play a role in plaque rupture (Fig. 2C). Finally, there was an inverse correlation between $CD4^+$ $CD28^{null}$/Treg ratio and cap-thickness ($R = -0.44$; $P = 0.002$) (Fig. 2D).

At multivariate analysis, $CD4^+$ $CD28^{null}$ T-cell frequency and $CD4^+$ $CD28^{null}$/Treg ratio were independently associated with RFC (OR 1.48, 95% CI 1.10–1.98, $P = 0.009$ and OR 1.10, 95% CI 1.02–1.19, $P = 0.008$, respectively). Best cut-off values at the ROC curve analysis were $CD4^+$ $CD28^{null}$ T-cell frequency ≥ 10.6% (sensitivity 100%, 95% CI 73.5–100%; specificity 88.9%, 95% CI 73.9–96.9) and $CD4^+$ $CD28^{null}$/Treg ratio ≥ 3.4% (sensitivity 91.7%, 95% CI 81.5–99.8%; specificity 83.3%, 95% CI 67.2–93.6).

## 4. Discussion

In the last decade, the advent of OCT has shed new light on our knowledge of the pathophysiological mechanisms of ACS. Previous *post-mortem* studies demonstrated that not only ruptured plaques, but also eroded plaques with intact fibrous cap could complicate with thrombus formation [17]. In the era of statin treatment, we are facing a gradual shift in the histological features of unstable plaque, from rupture to erosion. However, plaque rupture still represent the most common plaque morphology responsible for thrombosis and the most frequent substrate of myocardial infarction, especially among men [18].

An adaptive immune imbalance with a skewed T cell differentiation, oriented toward aggressive effector T cells and defective Tregs, have been documented in ACS patients with systemic inflammation [2,3]. $CD4^+$ $CD28^{null}$ T-cells represent a specific subset with proatherogenic and plaque-destabilizing properties [4,5], potentially involved in fibrous cap rupture and preferentially founded in unstable ruptured plaques [6].

In the present study, we observed a deeper T-cell perturbation in NSTEMI patients with RFC at OCT evaluation of the culprit lesion as compared with NSTEMI-IFC and SA patients, characterized by a significant expansion of aggressive $CD4^+$ $CD28^{null}$ T lymphocytes in the absence of an adequate Treg response, thus representing the failure to mount an effective counter regulatory response.

Moreover an inverse correlation between $CD4^+$ $CD28^{null}$/Treg ratio and cap thickness was observed, suggesting that inflammation could decisively contribute to plaque rupture. Our results are in line with previous evidence that patients with RFC had a higher plaque burden [16] and higher serum CRP and matrix metalloproteinase-9 levels [15], and identify for the first time a subset of patients with a specific adaptive immune system profile that might be associated to a more event-prone coronary artery plaque phenotype.

Finally, it is well known that ACS patients presenting with RFC as culprit lesion have a worse outcome, mainly due to recurrent analogue episodes of coronary instability [11]. Nevertheless, the clinical relevance of the adaptive immunity alterations investigated in this study was previously well elucidated by our group [9], by demonstrating that NSTE-ACS patients with this distinct immune system profile had a worse outcome, with a significantly higher incidence of new acute coronary events at 12-months follow-up. In the era of precision medicine, the knowledge of the specific immune signature of this sizeable proportion of patients could pave the way to a more tailored therapeutic approach [1,18].

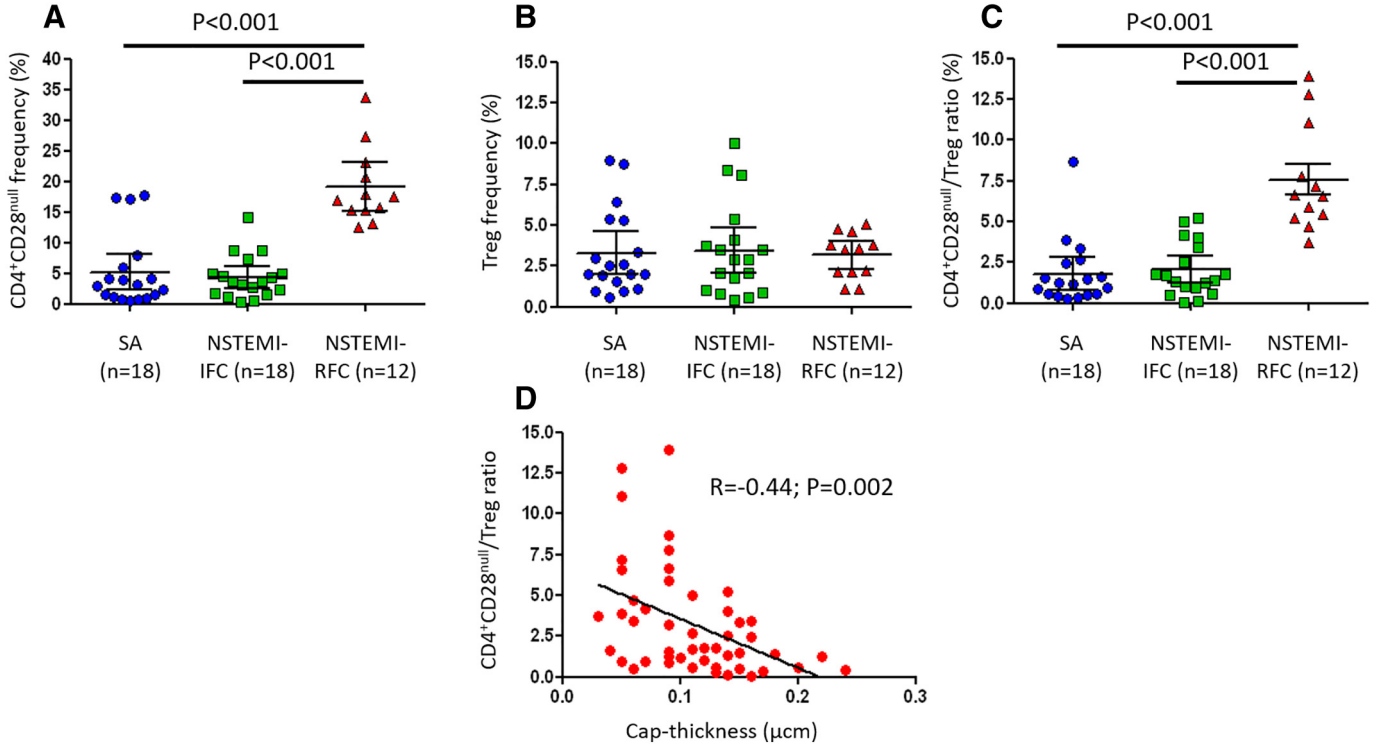


**Fig. 2.** $CD4^+$ $CD28^{null}$ T lymphocytes, Treg and $CD4^+$ $CD28^{null}$/Treg ratio in NSTEMI patients. A. $CD4^+$ $CD28^{null}$ lymphocytes frequency was significantly higher in NSTEMI-RFC patients as compared with NSTEMI-IFC and SA ($P < 0.001$ for all comparisons). B. Treg frequency did not show statistically significant difference among the three groups of study. C. $CD4^+$ $CD28^{null}$/Treg ratio was found significantly higher in NSTEMI-RFC patients as compared to NSTEMI-IFC and SA ($P < 0.001$ for all comparisons). Data are presented as single data points and mean 95% CI (12 NSTEMI-RFC patients, 18 NSTEMI-IFC patients, and 18 SA patients were included in the analysis). D. Correlation analysis with relative scatter graph and regression line showing a linear inverse correlation between $CD4^+$ $CD28^{null}$/Treg ratio and cap-thickness. Abbreviations. ACS = Acute Coronary Syndrome; NSTEMI-RFC = Non-ST Elevation Myocardial Infarction with evidence of Ruptured Fibrous Cap; NSTEMI-IFC = Non-ST Elevation Myocardial Infarction with evidence of Intact Fibrous Cap; SA = Stable Angina; Treg = Regulatory T lymphocytes; CI = Confidence Interval.

### 4.1. Study limitations

This is an observational prospective analysis, including a limited number of patients. No power calculation could be performed because of the lack of previous studies in this setting, thus the enrollment of patients in each group was arbitrary.

Patients were not matched for risk factors; however, no significant differences were observed to this regard.

T-cell count and function in peripheral blood not necessarily reflect what happens in the microenvironment of the unstable plaque; yet, we previously showed that $CD4^+$ $CD28^{null}$ T-cells infiltrate unstable plaques [6], where Treg represent a minority of T-cell population.

The association between transient adaptive immunity alterations and coronary thrombosis remains exploratory in nature, because a causal link could not be inferred. We could speculate that only alterations persisting after clinical stabilization of the disease might have a direct and causative role; on the contrary the direction of causality in the acute phase might only be clarified by Mendelian randomization or intervention trials. For this reason further prospective studies will be needed to unravel this important conundrum.

Our findings suggest an association between an imbalance in different subsets of T lymphocytes (i.e. $CD4^+$ $CD28^{null}$ and Treg) and a specific OCT feature, i.e. plaque rupture, but do not resolve the question of causality and they need to be validated in a separate prospective study.

### 4.2. Conclusions

Patients with NSTEMI presenting with RFC as culprit lesion at OCT evaluation have a specific perturbation of adaptive immunity, mostly involving -$CD4^+$ $CD28^{null}$ T lymphocytes and Tregs, compared with patients with IFC and SA. This specific imbalance of T-cells might play a key role in fibrous cap thinning, predisposing atherosclerotic plaque to rupture.

## Acknowledgments and sources of funding

This work was supported in part by the Catholic University of the Sacred Heart, Rome, Italy [Grant R4124500458 LINEA D.1 2016].

## Conflict of interest

None declared.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijcard.2018.08.101.

## References

[1] F. Crea, G. Liuzzo, Pathogenesis of acute coronary syndromes, J. Am. Coll. Cardiol. 61 (2013) 1–11, https://doi.org/10.1016/j.jacc.2012.07.064.
[2] D. Flego, G. Liuzzo, C.M. Weyand, F. Crea, Adaptive immunity dysregulation in acute coronary syndromes: from cellular and molecular basis to clinical implications, J. Am. Coll. Cardiol. 68 (2016) 2107–2117, https://doi.org/10.1016/j.jacc.2016.08.036.
[3] D. Flego, A. Severino, F. Trotta, et al., Increased PTPN22 expression and defective CREB activation impair regulatory T-cell differentiation in non-ST-segment elevation acute coronary syndromes, J. Am. Coll. Cardiol. 65 (2015) 1175–1186, https://doi.org/10.1016/j.jacc.2015.01.027.
[4] G. Liuzzo, S.L. Kopecky, R.L. Frye, et al., Perturbation of the T-cell repertoire in patients with unstable angina, Circulation 100 (1999) 2135–2139, https://doi.org/10.1161/01.CIR.100.21.2135.
[5] I.E. Dumitriu, E.T. Araguás, C. Baboonian, J.C. Kaski, $CD4^+$ $CD28^{null}$ T cells in coronary artery disease: when helpers become killers, Cardiovasc. Res. 81 (2009) 11–19, https://doi.org/10.1093/cvr/cvn248.
[6] G. Liuzzo, J.J. Goronzy, H. Yang, et al., Monoclonal T-cell proliferation and plaque instability in acute coronary syndromes, Circulation 101 (2000) 2883–2888, https://doi.org/10.1161/01.CIR.101.25.2883.
[7] G. Liuzzo, L.M. Biasucci, G. Trotta, et al., Unusual $CD4^+$ $CD28^{null}$ T lymphocytes and recurrence of acute coronary events, J. Am. Coll. Cardiol. 50 (2007) 1450–1458, https://doi.org/10.1016/j.jacc.2007.06.040.
[8] S. Giubilato, G. Liuzzo, S. Brugaletta, et al., Expansion of $CD4^+$ $CD28^{null}$ T-lymphocytes in diabetic patients: exploring new pathogenetic mechanisms of increased cardiovascular risk in diabetes mellitus, Eur. Heart J. 32 (2011) 1214–1226, https://doi.org/10.1093/eurheartj/ehq499.
[9] G. Liuzzo, R.A. Montone, M. Gabriele, et al., Identification of unique adaptive immune system signature in acute coronary syndrome, Int. J. Cardiol. 168 (2013) 564–567, https://doi.org/10.1016/j.ijcard.2013.01.009.
[10] G. Niccoli, G. Liuzzo, R.A. Montone, F. Crea, Advances in mechanisms, imaging and management of the unstable plaque, Atherosclerosis 233 (2014) 467–477, https://doi.org/10.1016/j.atherosclerosis.2014.01.036.
[11] G. Niccoli, R.A. Montone, L. Di Vito, et al., Plaque rupture and intact fibrous cap assessed by optical coherence tomography portend different outcomes in patients with acute coronary syndrome, Eur. Heart J. 36 (2015) 1377–1384, https://doi.org/10.1093/eurheartj/ehv029.
[12] A.J.S. Coats, L.G. Shewan, Statement on authorship and publishing ethics in the International Journal of Cardiology, Int. J. Cardiol. 153 (2011) 239–240, https://doi.org/10.1016/j.ijcard.2011.10.119.
[13] F. Prati, E. Regar, G.S. Mintz, et al., Expert review document on methodology, terminology, and clinical applications of optical coherence tomography: physical principles, methodology of image acquisition, and clinical application for assessment of coronary arteries and atherosclerosis, Eur. Heart J. 31 (2010) 401–415, https://doi.org/10.1093/eurheartj/ehp433.
[14] H. Jia, F. Abtahian, A.D. Aguirre, et al., In vivo diagnosis of plaque erosion and calcified nodule in patients with acute coronary syndrome by intravascular optical coherence tomography, J. Am. Coll. Cardiol. 62 (2013) 1748–1758, https://doi.org/10.1016/j.jacc.2013.05.071.
[15] G. Niccoli, R.A. Montone, L. Cataneo, et al., Morphological-biohumoral correlations in acute coronary syndromes: pathogenetic implications, Int. J. Cardiol. 171 (2014) 463–466, https://doi.org/10.1016/j.ijcard.2013.12.238.
[16] G. Scalone, G. Niccoli, H. Refaat, et al., Not all plaque ruptures are born equal: an optical coherence tomography study, Eur. Heart J. Cardiovasc. Imaging 18 (11) (2017) 1271–1277.
[17] E. Falk, M. Nakano, J.F. Bentzon, A.V. Finn, R. Virmani, Update on acute coronary syndromes: the pathologists' view, Eur. Heart J. 34 (2013) 719–728, https://doi.org/10.1093/eurheartj/ehs411.
[18] F. Crea, P. Libby, Acute coronary syndromes: the way forward from mechanisms to precision treatment, Circulation 136 (2017) 1155–1166, https://doi.org/10.1161/CIRCULATIONAHA.117.029870.