European Heart Journal (2016) **37**, 1550–1552
doi:10.1093/eurheartj/ehw036

EDITORIAL

Histopathological thrombus analysis in patients with stent thrombosis: what are the missing pieces in the puzzle?

Lorenz Räber*, Konstantinos C. Koskinas, and Stephan Windecker

Department of Cardiology, Bern University Hospital, Bern, Switzerland

Online publish-ahead-of-print 6 February 2016

This editorial refers to 'Histopathological evaluation of thrombus in patients presenting with stent thrombosis: a multicentre European study'†, by J. Riegger *et al.*, on page 1538.

Myocardial revascularization by means of percutaneous coronary interventions has importantly advanced treatment of patients with coronary artery disease (CAD), notably acute coronary syndromes. The transition from balloon angioplasty to initially bare metal stents (BMS), followed by early-generation and subsequently new-generation drug-eluting stents (DES), improved outcomes in terms of both safety and efficacy, particularly with regard to reducing the risk of in-stent restenosis.[1] Stent thrombosis (ST) is a rare but potentially devastating complication which is associated with a high incidence of acute myocardial infarction, death, and need for repeat revascularization. Previous studies have highlighted the multifactorial nature of ST, which has been linked to various stent- and lesion-related factors, patient-specific characteristics, and medication compliance. These insights translated into improvements in stent technology as well as adjunct antithrombotic therapy, which in turn led a considerable reduction of rates of ST in the current era of new-generation DES.[2,3] Notwithstanding, ST remains an important concern due to the accumulating long-term risk and the high case fatality.

Thrombus formation represents the final step of two intertwined pathways, blood coagulation and platelet aggregation, which ultimately result in thrombotic stent occlusion when occurring inside the stent or in its vicinity. While platelets assume a central role in the pathobiology of ST and antiplatelet agents have effectively reduced the risk of ST, the role of inflammatory and immune cells in the biology of intravascular thrombosis is increasingly appreciated. Chemokines released by thrombocytes induce the incorporation of innate immune cells (monocytes and neutrophils) into the developing thrombus; these cells, as well as eosinophils, precipitate fibrin formation and additional platelet activation, and they release intravascular tissue factor that further potentiates clot formation.[4] Identification of these cell lines within the stented vessel wall in autopsy studies[5] and histopathological analyses of thrombus aspirates[6–8] of patients with ST has been valuable in unravelling the underlying mechanisms.

Study findings

In this issue of the journal, Riegger *et al.* provide new evidence from the PRESTIGE registry,[9] an ambitious interdisciplinary consortium aiming to investigate ST systematically across several European institutions. Specimens from thrombus aspirates from a heterogeneous group of patients presenting with ST were analysed; most patients had incurred late ST (i.e. >30 days post-implantation) and were treated predominantly (66%) with DES. Thrombi were found to be heterogeneous, rich in platelets and fibrin fragments, and contained abundant leucocytes. Eosinophils and so-called neutrophil extracellular traps (NETs; extracellular, neutrophil-derived DNA matrices with recognized procoagulant function) were observed frequently, suggesting a role for innate immunity in the pathobiology of ST according to the authors. Notably, no differences in terms of thrombus composition (i.e. leucocytes, neutrophils, and eosinophils) were observed when comparing early vs. late ST or DES vs. BMS. In addition, no difference in NETs was noted. In view of the substantially different causes underlying the pathobiological processes involved in ST related to DES as compared with BMS or early as compared with very late ST, the results of the present study suggest that the information derived from thrombus aspirates is not specific enough to determine the cause of ST. The only trend which was observed concerned a higher proportion of eosinophils in durable polymer sirolimus-eluting stents (SES) and everolimus-eluting stents (EES; both with methacrylate as the polymer component), as compared with all other stent types, which is a confirmation of previous studies suggesting a device-specific hypersensitivity particularly after the use of durable polymer SES.[6,7] To determine

The opinions expressed in this article are not necessarily those of the Editors of the *European Heart Journal* or of the European Society of Cardiology.

† doi:10.1093/eurheartj/ehv419.

* Corresponding author. Department of Cardiology, Bern University Hospital, 3010 Bern, Switzerland. Tel: +41 31 632 09 29, Fax: +41 632 47 71, Email: lorenz.raeber@insel.ch

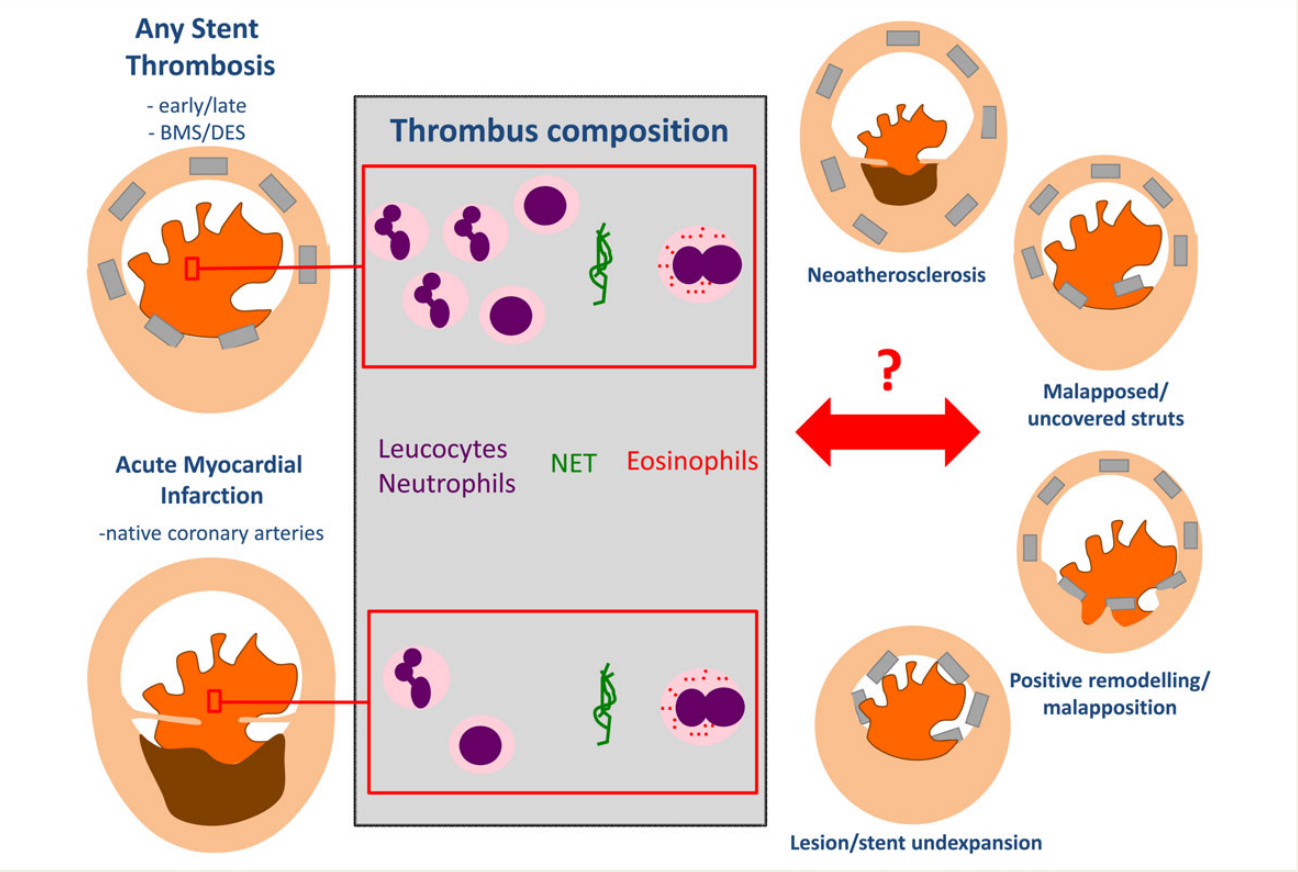

Figure 1 Schematic figure summarizing the main findings of the PRESTIGE study: (i) no difference in thrombus composition depending on stent type and implant duration; (ii) predominance of leucocytes/neturophils in stent thrombosis compared with acute myocardial infarction aspirates and similar eosinophilic cell counts. Whether various well-characterized causes of stent thrombosis (i.e. neoatherosclerosis, hypersensitivity, malapposed and uncovered stent struts, and underexpansion) display similar thrombus composition remains unaddressed by this study. BMS, bare metal stent; DES, drug-eluting stent; NET, neutrophil extracellular trap.

whether the thrombus aspirates in the case of ST feature a unique fingerprint, the investigators conducted a post-hoc comparison with aspirates obtained from patients with native vessel acute myocardial infarction (AMI) and observed a lower leucocyte cell count owing to a lower number of neutrophils, yet a similar amount of eosinophils and NETs was found.[9] This result represents one of the principal findings of PRESTIGE suggesting a predominance of inflammatory cells in ST compared with AMI aspirates (*Figure 1*).

Missing link with intracoronary imaging

The lack of differences in the composition of thrombus aspirates derived from ST related to DES as compared with BMS as well as according to time elapsed since stent implantation is notable in view of the multifactorial aetiology of ST. Histopathological studies and *in vivo* imaging investigations using intravascular ultrasound (IVUS) and optical coherence tomography (OCT) have established several lines of evidence. First, there are time-related differences regarding the causative mechanism, such that ST occurring acutely or early is closely related to mechanical abnormalities (dissections and stent underexpansion),[10] whereas very late ST (>1 year) develops on a background of long-term pathobiological processes involving the device *per se* and its interaction with the native vessel wall[11] (*Figure 1*). These mechanisms include malapposition (which either persists since the time of implantation or develops later on as a result of positive remodelling or vessel wall hypersensitivity reaction); neoatherosclerosis (a process of in-stent atheroma formation that correlates with natural disease progression[12]); and uncovered stent struts (an expression of delayed arterial healing largely related to the antiproliferative drug).[11] Secondly, device-related differences exist regarding the risk of ST, which are related to the eluted drug, drug/polymer coatings, and various characteristics of stent design.

One important limitation of the present study is the missing correlation of various aspects of thrombus composition with specific, imaging-defined mechanical stent abnormalities. Previous studies have demonstrated a direct correlation between the presence and extent of late malapposition after durable polymer SES implantation and the proportion of eosinophilic cells.[6] Along the same line, Yamaji and colleagues observed a prevailing expression of eosinophils in thrombus aspirates obtained from patients with very late ST, with angiographic evidence of persistent contrast staining- or IVUS-defined incomplete stent apposition.[7] A mechanistic link

between the cause of stent thrombosis and thrombus composition could not be corroborated by the PRESTIGE investigators in the absence of intracoronary imaging, although these investigations represent an important foundation of the registry.[9] Additional aspects remain unanswered, including the presence of atherosclerotic components such as macrophages, cholesterol crystals, or fibrous cap material, or markers of oxidative stress such as myeloperoxidase, hypothetically indicating the presence of neoatherosclerosis as an increasingly important cause of ST. Indeed, earlier studies by Yamaij and colleagues suggested that atherosclerotic components had an increasing prevalence in BMS patients with very late ST beyond 3 years, suggesting a close association with neoatherosclerosis.[8]

Therapeutic consequences

What are the clinical implications of PRESTIGE in the overarching goal to prevent ST? The authors postulate that based on their findings, pharmacological agents targeting immunothrombosis may represent potential preventive strategies. Indeed, prolonged and combined antiplatelet therapy (the current standard of care in the prevention of ST) inhibits the physiological haemostasis process, therefore inherently lowering the stent thrombosis risk at the cost of an increased bleeding risk. Selective treatments targeting immunity to prevent thrombosis appear attractive, but experimental evidence is limited to date and needs to be carefully weighed in the context of relevant side effects and costs of immunomodulatory therapies in other clinical settings[13] as well as the very low rates of ST in the current era of new-generation DES. Moreover, it is unlikely that systemic immunomodulatory treatments will effectively mitigate the thrombotic risk in the presence of a clear-cut mechanical stent abnormality such as stent underexpansion, major dissections or long segments of malapposition. Similarly, studies are currently underway that test the 'inflammatory hypothesis' of atherosclerosis by assessing the impact of systemic anti-inflammatory therapies on cardiovascular events; even if the much expected results of the CANTOS (NCT01327846) and CIRT trials (NCT01594333) are positive, it is unlikely that systemic inflammatory therapies will replace lipid-lowering therapies which prevent lipid accumulation within the vessel wall, i.e. the initial step of atherogenesis that leads to inflammatory reactions within the plaque.

As our understanding of the mechanisms of ST is growing, efforts will continue to focus on improving modifiable device- and implantation-related triggers of the thrombotic complication. Refinements of new-generation DES have focused on their composition, the thickness of the polymer, the material of the stent platform, stent geometry, and strut thickness, as well as the selection and dosage of antiproliferative agents. Optimal device implantation also appears to be essential. In this respect, the role of intracoronary imaging to guide and optimize the procedure in selected patients is established[14] and is expected to become even more prominent to improve outcomes in the emerging era of bioresorbable vascular scaffolds.[15]

Whether the compositional aspect of ST aspirates will ever prove to have sufficient specifity to inform the clinician on the underlying pathomechanism still remains an open question, and the PRESTIGE investigators should be encouraged to add the missing pieces to the puzzle, so that clinical utility may be an option one day.

Conflict of interest: L.R. and S.W. receive speaker fees and research grant support by St. Jude Medical, Switzerland.

References

1. Windecker S, Stortecky S, Stefanini GG, da Costa BR, Rutjes AW, Di Nisio M, Silletta MG, Maione A, Alfonso F, Clemmensen PM, Collet JP, Cremer J, Falk V, Filippatos G, Hamm C, Head S, Kappetein AP, Kastrati A, Knuuti J, Landmesser U, Laufer G, Neumann FJ, Richter D, Schauerte P, Sousa Uva M, Taggart DP, Torracca L, Valgimigli M, Wijns W, Witkowski A, Kolh P, Jüni P. Revascularisation versus medical treatment in patients with stable coronary artery disease: network meta-analysis. *BMJ* 2014;**348**:g3859.
2. Räber L, Magro M, Stefanini GG, Kalesan B, van Domburg RT, Onuma Y, Wenaweser P, Daemen J, Meier B, Jüni P, Serruys PW, Windecker S. Very late coronary stent thrombosis of a newer-generation everolimus-eluting stent compared with early-generation drug-eluting stents: a prospective cohort study. *Circulation* 2012;**125**:1110–1121.
3. Stefanini GG, Byrne RA, Serruys PW, de Waha A, Meier B, Massberg S, Jüni P, Schömig A, Windecker S, Kastrati A. Biodegradable polymer drug-eluting stents reduce the risk of stent thrombosis at 4 years in patients undergoing percutaneous coronary intervention: a pooled analysis of individual patient data from the ISAR-TEST 3, ISAR-TEST 4, and LEADERS randomized trials. *Eur Heart J* 2012;**33**:1214–1222.
4. Semple JW, Italiano JE, Freedman J. Platelets and the immune continuum. *Nat Rev Immunol* 2011;**11**:264–274.
5. Otsuka F, Vorpahl M, Nakano M, Foerst J, Newell JB, Sakakura K, Kutys R, Ladich E, Finn AV, Kolodgie FD, Virmani R. Pathology of second-generation everolimus-eluting stents versus first-generation sirolimus- and paclitaxel-eluting stents in humans. *Circulation* 2014;**129**:211–223.
6. Cook S, Ladich E, Nakazawa G, Eshtehard P, Neidhart M, Vogel R, Togni M, Wenaweser P, Billinger M, Seiler C, Gay S, Meier B, Pichler WJ, Jüni P, Virmani R, Windecker S. Correlation of intravascular ultrasound findings with histopathological analysis of thrombus aspirates in patients with very late drug-eluting stent thrombosis. *Circulation* 2009;**120**:391–399.
7. Yamaji K, Kubo S, Inoue K, Kadota K, Kuramitsu S, Shirai S, Ando K, Nobuyoshi M, Mitsudo K, Kimura T. *PLoS One* 2014;**9**:e113870.
8. Yamaji K, Inoue K, Nakahashi T, Noguchi M, Domei T, Hyodo M, Soga Y, Shirai S, Ando K, Kondo K, Sakai K, Iwabuchi M, Yokoi H, Nosaka H, Nobuyoshi M, Kimura T. Bare metal stent thrombosis and in-stent neoatherosclerosis. *Circ Cardiovasc Interv* 2012;**5**:47–54.
9. Riegger J, Byrne RA, Joner M, Chandraratne S, Gershlick A, ten Berg JM, Adriaenssens T, Guagliumi G, Godschalk TC, Neumann F, Trenk D, Feldman LJ, Steg PG, Desmet W, Alfonso F, Goodall AH, Wojdyla R, Dudek D, Philippi V, Opinaldo S, Titova A, Malik N, Cotton J, Jhagroe DA, Heestermans AA, Sinnaeve P, Vermeersch P, Valina C, Schulz C, Kastrati A, Massberg S. Histopathological evaluation of thrombus in patients presenting with stent thrombosis: a multicentre European study. *Eur Heart J* 2016;**37**:1538–1549.
10. Parodi G, La Manna A, Di Vito L, Valgimigli M, Fineschi M, Bellandi B, Niccoli G, Giusti B, Valenti R, Cremonesi A, Biondi-Zoccai G, Prati F. Stent-related defects in patients presenting with stent thrombosis: differences at optical coherence tomography between subacute and late/very late thrombosis in the Mechanism Of Stent Thrombosis (MOST) study. *EuroIntervention* 2013;**9**:936–944.
11. Taniwaki M, Radu MD, Zaugg S, Amabile N, Garcia-Garcia HM, Yamaji K, Jørgensen E, Kelbæk H, Pilgrim T, Caussin C, Zanchin T, Veugeois A, Abildgaard U, Jüni P, Cook S, Koskinas KC, Windecker S, Räber L. Mechanisms of very late drug-eluting stent thrombosis assessed by optical coherence tomography. *Circulation* 2016. [Epub ahead of print].
12. Taniwaki M, Windecker S, Zaugg S, Stefanini GG, Baumgartner S, Zanchin T, Wenaweser P, Meier B, Jüni P, Räber L. The association between in-stent neoatherosclerosis and native coronary artery disease progression: a long-term angiographic and optical coherence tomography cohort study. *Eur Heart J* 2015;**36**:2167–2176.
13. Robert C, Schachter J, Long GV, Arance A, Grob JJ, Mortier L, Daud A, Carlino MS, McNeil C, Lotem M, Larkin J, Lorigan P, Neyns B, Blank CU, Hamid O, Nateus C, Shapira-Frommer R, Kosh M, Zhou H, Ibrahim N, Ebbinghaus S, Ribas A. Pembrolizumab versus Ipilimumab in advanced melanoma. *N Engl J Med* 2015;**372**:2521–253.
14. Windecker S, Kolh P, Alfonso F, Collet JP, Cremer J, Falk V, Filippatos G, Hamm C, Head SJ, Jüni P, Kappetein AP, Kastrati A, Knuuti J, Landmesser U, Laufer G, Neumann FJ, Richter DJ, Schauerte P, Sousa Uva M, Stefanini GG, Taggart DP, Torracca L, Valgimigli M, Wijns W, Witkowski A. 2014 ESC/EACTS Guidelines on myocardial revascularization: the Task Force on Myocardial Revascularization of the European Society of Cardiology (ESC) and the European Association for Cardio-Thoracic Surgery (EACTS). *Eur Heart J* 2014;**35**:2541–2619.
15. Räber L, Brugaletta S, Yamaji K, O'Sullivan CJ, Otsuki S, Koppara T, Taniwaki M, Onuma Y, Freixa X, Serruys P, Joner M, Sabaté M. Very late scaffold thrombosis: intracoronary imaging, histopathological and spectroscopy findings. *J Am Coll Cardiol* 2015;**66**:1901–1914.