Enhanced expression of haemoglobin scavenger receptor in accumulated macrophages of culprit lesions in acute coronary syndromes

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Aims Effective clearance of extracellular haemoglobin (Hb) is thought to limit systemic oxidative heme toxicity, which is presumed to contribute to the pathogenesis of plaque instability. We immunohistochemically examined the relationship between intraplaque haemorrhage, 4-HNE (4-hydroxy-2-nonenal), an index of lipid peroxidation, and the Hb scavenger receptor (CD163) using coronary atherectomy specimens from 74 patients with stable angina pectoris (SAP, \(n=39\)) or unstable angina pectoris (UAP, \(n=35\)).

Methods and results Atherectomy samples were stained with antibodies against glycophorin A (a protein specific to erythrocyte membranes), CD31, 4-HNE, and CD163. Quantitative analysis demonstrated that glycophorin A-positive areas, 4-HNE-positive macrophage score, and CD163-positive macrophage score in UAP patients were significantly higher (glycophorin A, \(P<0.0001\); 4-HNE-positive macrophage score, \(P<0.0001\); CD163-positive macrophage score, \(P<0.0005\)) than in SAP patients. The percentage of the glycophorin A-positive area showed a significant positive correlation with the number of CD31-positive microvessels and the 4-HNE-positive macrophage score (microvessels, \(R=0.59, P<0.0001\); 4-HNE, \(R=0.59, P<0.0001\)). Moreover, the CD163-positive macrophage score was positively correlated with glycophorin A-positive area and the 4-HNE-positive macrophage score (glycophorin A, \(R=0.58, P<0.0001\); 4-HNE, \(R=0.53, P<0.0001\)).

Conclusion These findings suggest a positive association among intraplaque haemorrhage, enhanced expression of Hb scavenger receptor, and lipid peroxidation in human unstable plaques.

Keywords Atherosclerosis ● Coronary disease ● Angina ● Lipoproteins

Introduction The progression of atherosclerosis is a complex phenomenon and the conversion of a stable, asymptomatic plaque to an unstable plaque that may ultimately cause plaque rupture or erosion with mural thrombus formation involves many processes. Previous studies have shown that inflammation and oxidative stress in coronary atherosclerotic lesions contribute to rapidly progressive plaque destabilization. Inflammatory phenomena within vulnerable plaques might explain plaque rupture or erosion and subsequent thrombosis, resulting in total vessel occlusion and myocardial infarction. Most studies demonstrated that macrophages and T lymphocytes are the dominant types of inflammatory cells in human coronary unstable plaques, such as ruptured or eroded...
Haemoglobin receptor expression in unstable plaques

In plaques, we previously demonstrated that elevated plasma oxidized low-density lipoprotein (ox-LDL) levels are related to plaque instability. Moreover, we demonstrated the infiltration of neutrophils with an increased activity of the pro-oxidant enzyme, myeloperoxidase, in the culprit lesions of patients with unstable angina pectoris (UAP) and showed that this plays a role in destabilizing atherosclerotic plaques.

Recent studies have also demonstrated that intraplaque haemorrhage is associated with the development of atherosclerotic lesions and plaque instability. There is evidence in patients who died suddenly from acute coronary syndromes that intraplaque haemorrhage may represent a potent atherogenic stimulus contributing to the deposition of free cholesterol, macrophage infiltration, and enlargement of the necrotic core. In cells oxidized stress, there is increased production of 4-hydroxy-2-nonenal (4-HNE), a major product of lipid peroxidation. Haemoglobin (Hb)-induced oxidative damage is provided by the protein haptoglobin (Hp), which rapidly and irreversibly binds to extracorporeal Hb, forming an Hp–Hb complex. In atherosclerotic plaques, the only route for clearance of the Hp–Hb complex is the macrophage, which is mediated by the membrane receptor CD163. CD163 is recognized as the specific Hb scavenger receptor and expressed by resident tissue macrophages. It is hypothesized that the CD163 plays a role in the control of inflammatory processes, probably controlling the expression of pro-inflammatory or anti-inflammatory cytokines. The expression of CD163 has been shown to be enhanced by anti-inflammatory mediators, such as interleukin-10 and glucocorticoids, suggesting that CD163 expression is related to anti-inflammatory functions. Other investigations also reported a role of CD163 in pro-inflammatory activation of macrophages. However, the correlation of intraplaque haemorrhage, 4-HNE, an index of lipid peroxidation, and the Hb scavenger receptor (CD163) in human coronary unstable plaques has not been previously reported. Therefore, we investigated the immunolocalization of intraplaque haemorrhage, 4-HNE, and CD163 in coronary atherectomy specimens taken from the culprit lesions responsible for stable angina pectoris (SAP) and UAP.

Methods

The study was approved by the hospital’s Ethics Committee, and informed consent was obtained from all patients before the study.

Patients

Coronary atherectomy specimens were obtained by directional coronary atherectomy (DCA) performed between April 2004 and March 2007, from the culprit lesions in 74 patients who presented with either SAP (n = 39) or UAP (n = 35). Twenty of 74 patients were already reported in another study. The UAP patients consisted of 18 patients in Braunwald’s Class I, 3 patients in Class II, and 14 in Class III undergoing percutaneous coronary intervention for a single primary lesion at Osaka City General Hospital, Osaka (restenosis specimens, vein graft specimens, and specimens from patients who required multiple interventions at the same time were excluded). Regarding the clinical circumstances, 31 patients were Class B and 4 patients were Class C. In the 35 UAP patients, serum CK-MB levels were 9.6 ± 4.1 IU/mL, and 6 of 35 patients (17%) had elevated cardiac troponin T levels (more than 0.1 ng/mL). The baseline ECG findings were as follows: 18 patients (51%) of 35 UAP showed ST-segment depression (more than 0.1 mV) or T-wave inversion, or both, and two patients (6%) showed ST-segment elevation. The culprit lesion was identified on the basis of clinical, ECG, and angiographic data. Patients with a culprit lesion that could not be identified were excluded from the study. Patients were selected for DCA using strictly defined angiographic criteria: a proximal located eccentric culprit lesion in a non-tortuous coronary artery more than 3 mm in diameter. Atherectomy was performed with a femoral approach using an 8F arterial sheath and guide catheter, and a DCA catheter (AtheroCath-GTO, Devices for Vascular Intervention, Inc.) of appropriate size to produce an approximate device-to-artery ratio of 1.1:1. Immediately after atherectomy, the tissue specimens were carefully oriented along their longest axis, snap-frozen, and stored at –80°C. The snap-frozen samples obtained by DCA were subsequently serially sectioned to produce sections of 5 μm in thickness, and then fixed in acetone. Each first and second section was stained with haematoxylin–eosin stain and Berlin blue stain for iron, respectively. The other sections were used for immunohistochemical staining.

Coronary angiographic analysis

Angiography was performed so that each lesion could be viewed from at least two angles. In all patients, off-line quantitative coronary angiography (QCA) was conducted with the view revealing the highest degree of stenosis. Calculations were performed using the Cardiovascular Measurement System (CMS-MEDIS Medical Imaging System, Leiden, The Netherlands) by an investigator who was blinded to the study design. Pre-intervention minimal lumen diameter (MLD) and diameter stenosis (DS) were calculated. Coronary stenoses were viewed in two orthogonal projections and classified as ‘complex’ or ‘smooth’ based on the Ambrose classification.

Immunohistochemistry

Single immunostaining

The cellular components were analysed by the use of monoclonal antibodies against smooth muscle cell actin (1A4, DAKO, Glostrup, Denmark), macrophages (EBM11, DAKO), a protein specific to erythrocyte membranes (Glycophorin A, DAKO), the macrophage Hb scavenger receptor (CD163, Santa Cruz, CA, USA), and 4-HNE (NOF corporation, Tokyo, Japan). To identify neutrophils, the following antibodies were used CD66b (80H3, Beckman Coulter, Fullerton, CA, USA), elastase (NP57, DAKO), and myeloperoxidase (MPO-7, DAKO). Microvessels of the tissue sections were assessed using antibodies for CD31 (DAKO), CD34 (DAKO), and von Willebrand factor (vWF) (DAKO). Non-immune mouse IgG serum (DAKO) served as a negative control. Sections were incubated at 4°C overnight or for 1 h at room temperature, and then subjected to a three-step staining procedure, using the streptavidin–biotin complex method for detection. Peroxidase activity was visualized with 3-amin-9-ethylcarbazole (10 min, room temperature), and the sections were faintly counterstained with haematoxylin.

Double immunostaining

Simultaneous identification of smooth muscle cells and macrophages was carried out using two primary antibodies to different IgG subclass proteins (1A4/CD68 or HAM56), as reported previously. The enzynatic activity of β-galactosidase for 1A4 was visualized as turquoise (BioGenex Kit, BioGenex), whereas alkaline phosphatase for CD68 was visualized as red (New Fuchsin Kit, DAKO). We also performed
double immunostaining for macrophages (CD68) and 4-HNE or CD163 using modifications of procedures reported previously. For double immunostaining, alkaline phosphatase was visualized with fast blue BB (blue: CD68) and peroxidase with 3-amino-9-ethylcarbazole development (red: 4-HNE or CD163).

Quantitative methods
The tissue area occupied by immunostained macrophages, glycophorin A, CD163-positive cells, and 4-HNE was quantified using computer-aided planimetry and expressed as a percentage of the total surface area of the tissue section. In addition, on the basis of these quantifications, a ‘4-HNE-positive macrophage score’ was calculated as follows: 4-HNE-positive macrophage score = 4-HNE-positive area/macrophage-positive area. In addition, a ‘CD163-positive macrophage score’ was also calculated as follows: CD163-positive macrophage score = CD163-positive area/macrophage-positive area. The number of microvessels was defined as follows: the number of CD31-positive endothelial cells was counted in the entire tissue sections and expressed as the number of cells per mm² of the tissue. The morphometric analysis was performed by a single investigator who was blinded to the patients’ characteristics and histological classifications. Data are shown as mean ± SD. The two groups of patients (SAP and UAP) were compared by a Mann–Whitney U test in all circumstances. Categorical variables were compared by use of a χ² test or Fisher’s exact test. Values of P < 0.05 were considered significant.

Results
Clinical presentation and angiographic findings
Patient characteristics are shown in Table 1. There were no significant differences between the two groups with respect to age, gender, risk factors, serum levels of total cholesterol, HDL cholesterol, LDL cholesterol, or triglycerides, and medical therapy at hospital admission. In contrast, mean leucocyte counts, mean neutrophil counts, and serum high sensitivity (hs) C-reactive protein levels were significantly higher in UAP patients than in SAP patients (leucocyte counts, P = 0.013; neutrophil counts, P = 0.022; hs-C-reactive protein, P = 0.031). Regarding QCA analysis, there were no significant differences in pre-procedure MLD or DS between the two groups. At the site of target lesion, ‘complex lesion’ was found in 4 of 39 patients (10%) with SAP and in 22 of 35 (63%) with UAP (P < 0.0001).

<table>
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<tr>
<th>Table 1 Clinical and angiographic features of patients who underwent directional coronary atherectomy</th>
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<td>SAP (n = 39)</td>
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<td>Age (years)</td>
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Values are mean ± SD or percentages; SAP, stable angina pectoris; UAP, unstable angina pectoris; HDL, high-density lipoprotein; LDL, low-density lipoprotein; QCA, quantitative coronary angiography; MLD, minimal lumen diameter; DS, diameter stenosis.
Histological findings

In the culprit lesions of patients with SAP, 18 of 39 lesions (46%) contained foci of macrophages, and only 2 of 39 lesions (5%) contained neutrophils. In contrast, all 35 lesions obtained from patients with UAP contained macrophages, and 20 lesions (57%) showed distinct neutrophil infiltration. In the culprit lesions of patients with UAP, 28 of 35 lesions (80%) contained abundant erythrocytes and 23 of 35 lesions (66%) contained iron deposits. Most of the iron deposits were found in macrophage-rich regions. In the lesions obtained from patients with UAP, abundant CD163-positive macrophages were also seen in the iron deposits and glycophorin A-positive areas. In addition, 4-HNE-positive macrophages were found in these lesions (Figure 1). In contrast, in the lesions from patients with SAP, 15 of 39 lesions (38%) contained foci of erythrocytes, and only 10 of 39 lesions (26%) showed iron deposits. There were only a few 4-HNE- or CD163-positive macrophages in the lesions from patients with SAP (Figure 1). Regarding the presence of microvessels, 29 of 35 lesions (83%) showed the presence of microvessels in the lesions of patients with UAP, whereas only 12 of 39 lesions (31%) obtained from patients with SAP contained microvessels (Figure 2). The CD163 antibody stained a subpopulation of the macrophages. Double immunostaining for CD163 and macrophages revealed that the vast majority of macrophages expressed CD163 in the lesions of patients with UAP, whereas only a few CD163-positive macrophages were present in the lesions of patients with SAP (Figure 3A). In addition, double immunostaining for 4-HNE and macrophages revealed that the vast majority of 4-HNE-positive cells were macrophages in the lesions of patients with UAP (Figure 3B). In contrast, in the lesions of patients with SAP, 4-HNE positivity was sparse, and when present, only a few macrophages were positive for 4-HNE.

Morphometric analysis

The percentages of the macrophage-positive area, the glycophorin A-positive area, and the iron-positive area were significantly higher (macrophages, 4(HNE)>0.0001; glycophorin A, 4(HNE)>0.0001; iron, 4(HNE)>0.005) in UAP than in SAP patients (Figure 4). The number of CD31-positive microvessels was significantly higher (4(HNE)>0.0001)

Figure 1
Micrographs of an atherectomy specimen obtained from a culprit lesion in a patient with UAP (A–E). (A) Fragment of atherosclerotic plaque tissue. Double immunostaining (smooth muscle cell, turquoise/macrophage, red) reveals abundant macrophages. (B) The adjacent section stained with anti-glycophorin A antibody reveals the presence of abundant glycophorin A-positive cells. (C) The section with Berlin blue staining shows iron deposits in a macrophage-rich region (arrows). (D) The anti-CD163 antibody shows that most macrophages are positive for CD163. (E) The anti-4-HNE antibody shows a large number of 4-HNE-positive macrophages. Micrographs of an atherectomy specimen obtained from a culprit lesion in a patient with SAP (F–J). (F) Fragment of atherosclerotic plaque tissue. Double immunostaining (smooth muscle cell, turquoise/macrophage, red) shows scattered macrophages in the lesion. (G) The adjacent section stained with anti-glycophorin A antibody reveals that there are no glycophorin A-positive cells. (H) The section with Berlin blue staining shows no iron deposits. (I) The anti-CD163 antibody shows that the scattered macrophages are negative for CD163. (J) The anti-4-HNE antibody shows that there are no 4-HNE-positive macrophages. Bar: A–J, 50 μm.
of 4-HNE-positive cells are macrophages. Bar: A and B, 100 μm.

Double immunostaining for macrophages (blue) and CD163 (red) reveals that most cells show double staining (purple), indicating that the vast majority of macrophages expressed CD163. (B) Double immunostaining for macrophages (blue) and 4-HNE (red) reveals that most cells show double staining (purple), indicating that the vast majority of 4-HNE-positive cells are macrophages. Bar: A and B, 100 μm.

Figure 2

Figure 3

Discussion

To the best of our knowledge, this is the first report to demonstrate the colocalization of Hb scavenger receptor (CD163), intraplaque haemorrhage, and 4-HNE, an oxidative stress-related molecule, in coronary atherectomy specimens taken from the culprit lesions responsible for SAP and UAP.

Pasterkamp and Virmani demonstrated that the erythrocyte is a potential component in atheromalous lesion formation, and suggested that when red blood cells undergo denaturation, the recognition of red cell membrane remnants by macrophages and subsequent internalization of membrane originating lipids may provide another substrate for foam cell formation. Moreover, it was shown in rabbits that a larger amount of both glycophorin A and iron was associated with larger necrotic cores and greater macrophage infiltration and that the crystallization of cholesterol from erythrocyte membranes may incite a foreign-body reaction. Moreover, a recent MRI study by Takaya et al. showed that intraplaque haemorrhage stimulates the progression of carotid atherosclerotic plaques. Taken together, these previous results suggest that the rapid accumulation of erythrocyte membranes causes an acute change in the plaque substrate characterized by increased free cholesterol within the core and excessive macrophage infiltration. In addition, neoangiogenesis is closely associated with plaque progression and is likely the primary source of intraplaque haemorrhage at sites of microvessel incompetence. The focal accumulation of T cell- and macrophage-derived angiogenic factors contributes to arborization of the vaso vasorum around the necrotic core, the formation of immature vessels, and the loss of the basement membrane around functional capillaries. In this study, the percentage of the glycophorin A-positive area showed a significant positive correlation with the number of CD31-positive microvessels and the 4-HNE-positive macrophage score (glycophorin A vs. microvessels, R = 0.59, P < 0.0001; glycophorin A vs. 4-HNE, R = 0.59, P < 0.0001, Figure 5A and B). Moreover, the CD163-positive macrophage score was positively correlated with the glycophorin A-positive area and the 4-HNE-positive macrophage score (CD163 vs. glycophorin A, R = 0.58, P < 0.0001; CD163 vs. 4-HNE, R = 0.53, P < 0.0001, Figure 5C and D).

In UAP and SAP patients, the percentage of the glycophorin A-positive area showed a significant positive correlation with the number of CD31-positive microvessels and the 4-HNE-positive macrophage score (glycophorin A vs. microvessels, R = 0.59, P < 0.0001; glycophorin A vs. 4-HNE, R = 0.59, P < 0.0001, Figure 5A and B). Moreover, the CD163-positive macrophage score was positively correlated with the glycophorin A-positive area and the 4-HNE-positive macrophage score (CD163 vs. glycophorin A, R = 0.58, P < 0.0001; CD163 vs. 4-HNE, R = 0.53, P < 0.0001, Figure 5C and D).
Figure 4 Graphs showing the macrophage-, glycophorin A-, and iron-positive areas expressed as a percentage of the total surface area, the number of microvessels/mm², the 4-HNE-positive macrophage score, and the CD163-positive macrophage score, in the atherectomy specimens obtained from the culprit lesions in patients with SAP or UAP.

Figure 5 (A and B) Graphs showing that the percentage of the glycophorin A-positive area is positively correlated with the number of microvessels and the 4-HNE-positive macrophage score (glycophorin A vs. microvessels, R = 0.59, P < 0.001; glycophorin A vs. 4-HNE, R = 0.59, P < 0.0001). (C and D) Graphs showing that the CD163-positive macrophage score is positively correlated with glycophorin A-positive area and the 4-HNE-positive macrophage score (CD163 vs. glycophorin A, R = 0.58, P < 0.0001; CD163 vs. 4-HNE, R = 0.53, P < 0.0001).
and iron, suggesting that phagocytosis of erythrocytes may contribute to the formation of foam cells. Similar lipid-containing cells, expressing both ceroid and inducible nitric oxide synthesis, have been generated in an atherosclerosis-free setting by incubating murine macrophages with oxidized erythrocytes. Moreover, erythropagocytosis as a result of microhaemorrhage may have additional consequences: the iron accumulated from the breakdown of Hb can act as a catalyst in the formation of free radicals, which may contribute to the modification of low-density lipoprotein cholesterol and cell death. These findings suggest that there may be a positive association between intraplaque haemorrhage and oxidative stress.

In this study, the percentages of the erythrocyte-positive area and the 4-HNE-positive macrophage score were significantly higher in atherectomy specimens from UAP than those from SAP patients. The increase in the percentage of the erythrocyte-positive area varied directly with the 4-HNE-positive macrophage score. Moreover, after erythrocyte membrane lysis, extracorpuscular Hb can induce oxidative tissue damage by virtue of its heme iron, with subsequent production of reactive oxygen species (ROS). Extracorpuscular Hb can also activate the proinflammatory transcription factor NF-κB, leading to inflammation and angiogenesis. Moreno et al. showed that the primary defence mechanism against Hb-induced oxidative damage is provided by the protein Hp, which rapidly and irreversibly binds to extracorpuscular Hb, forming an Hp–Hb complex. Effective clearance of extracellular Hb is thought to limit systemic oxidative heme toxicity, which is presumed to contribute to the pathogenesis of plaque instability. Cell-free Hb is tightly bound to Hp and subsequently cleared by CD163. Hb that is not bound to Hp can also be cleared via CD163. This Hp-independent, low-affinity Hb binding and uptake by CD163 is thought to be the predominant macrophage Hb clearance pathway after depletion of plasma Hp or during massive Hb release after erythrocyte extravasation on tissue injury. CD163 is expressed by resident tissue macrophages, and particularly high levels of CD163 have been detected in infiltrating monocytes during the resolution phase of inflammatory reactions. In this study, the CD163-positive macrophage score was significantly higher in UAP than in SAP patients. Moreover, abundant CD163-positive macrophages were seen in iron deposits or glycophorin A-positive areas in UAP patients compared with SAP patients. Additionally, the CD163-positive macrophage score was positively correlated with the 4-HNE-positive macrophage score, a major product of lipid peroxidation, and glycophorin A-positive area. These findings suggest that extracorpuscular Hb can induce oxidative tissue damage by virtue of its heme iron, with a subsequent production of ROS after erythrocyte membrane lysis in UAP patients.

Previously, we demonstrated that ox-LDL-positive macrophages were significantly higher in atherectomy specimens from patients with UAP than with SAP. Moreover, our previous study demonstrated that an infiltration of myeloperoxidase, a strong pro-oxidant enzyme released from activated neutrophils, occurs in the culprit lesions of UAP patients. Fernandez et al. previously

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**Figure 6** Schematic representation of the association among intraplaque haemorrhage, expression of haemoglobin scavenger receptor (CD163), and lipid peroxidation (4-HNE) in stable and unstable plaques in human coronary atherosclerotic lesions. The positive association among extravasation of red blood cells from leaky microvessels leading to intraplaque haemorrhage, enhanced expression of haemoglobin scavenger receptor CD163, and lipid peroxidation in macrophages may be an integral part of plaque progression associated with plaque inflammation in human unstable plaques.
demonstrated that CD163 is expressed on macrophages in atherosclerotic plaques in rabbits, and free Hb promotes atherogenesis by oxidizing LDL. In this study, the increase in the percentage of the erythrocyte-positive area and CD163-positive macrophage score showed a significant positive correlation with the 4-HNE-positive macrophage score. These findings suggest that intraplaque haemorrhage plays an important role in promoting the oxidative potential of ROS, which may lead to plaque instability and the development of acute coronary syndromes.

Study limitation
Coronary atherectomy specimens provide a unique source of plaque tissue because they make it possible to correlate plaque biology with the clinical status of the patient. We did not mention the extent of calcification in SAP and UAP patients by fluoroscopy. Although all patients underwent coronary angiography, the results were only used to determine the presence or absence of calcification, not the extent. This is due to the difficulties associated with determining the actual amount of calcification using coronary angiogram. In addition, we did not mention the amount of tissue retrieved from each sample. Since only part of the entire plaque was excised, a sampling bias must be considered in studies based on the examination of these specimens. Nevertheless, this potential limitation does not alter the conclusion regarding the association between intraplaque haemorrhage and plaque destabilization.

Conclusions
The present study revealed a positive association among extravasation of red blood cells from leaky microvessels leading to intraplaque haemorrhage, enhanced expression of Hb scavenger receptor CD163, and lipid peroxidation in macrophages in human coronary atherosclerotic plaques (Figure 6). This suggests that intraplaque haemorrhage may lead to increased oxidative stress and may contribute to plaque instability.

Acknowledgements
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Conflict of interest: none declared.

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‘Valve-in-valve’ implantation in a patient with degenerated aortic bioprosthesis and severe regurgitation

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A 70-year-old man was admitted due to dyspnoea (NYHA class III). In 1989, he underwent an aortic valve replacement using a Carpentier—Edwards bioprosthesis no. 21 and a left internal mammary artery—left anterior descending coronary bypass. In 2003, a laryngectomy and post-operative radiotherapy were performed because of laryngeal cancer. He then decided to stop smoking. At admission, blood pressure was 121/50 mmHg, and a murmur and peripheral signs of severe aortic regurgitation were noted. Transthoracic and transoesophageal echocardiography showed degeneration of the bioprosthesis with severe regurgitation due to cusp prolapse, left ventricular ejection fraction of 50%, and systolic pulmonary artery pressure at 60 mmHg (Panel A).

The coronary angiogram showed three-vessel disease with a patent graft.

The patient was formally denied surgical valve replacement because of high risk of mediastinitis due to laryngostomy and the impossibility of double thoracotomy due to radiation skin disease. In addition, the mortality risk predicted by the EuroSCORE was 35%.

The indication for trans-catheter aortic valve implantation was retained on a compassionate basis. After informed consent, a Core-Valve® Revalving System was successfully implanted into the Carpentier—Edwards bioprosthesis via femoral access (Panels B and C).

The hospital course was uncomplicated, with immediate functional improvement. At discharge, transthoracic echocardiography confirmed the adequate position of the prosthesis, the absence of residual regurgitation (Panel D), a left ventricular ejection fraction of 50%, and a systolic pulmonary artery pressure at 35 mmHg.

Despite the limited current experience, this case of ‘valve-in-valve’ implantation suggests that the technique is feasible and might be an attractive alternative to surgery for selected high-risk patients with severe dysfunction of degenerated bioprosthesis.

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