CASE REPORT

Visible-free cholesterol crystal emboli adjacent to microinfarcts in myocardial capillaries and arterioles on H&E-stained frozen sections of an autopsied patient

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SUMMARY

The number of released free cholesterol crystal emboli (fCCE) and their role during percutaneous coronary intervention (PCI) in acute coronary syndrome (ACS) have not been documented yet. Furthermore, fCCE manifesting in the coronary lumen following plaque rupture has been historically overlooked owing to the standard tissue preparation for light microscopy which uses ethanol as a dehydrating agent that can dissolve fCCE, leaving behind empty tissue. In this case report, we evaluated fCCE released during PCI for ACS and their relationship with myocardial injury and coronary artery obstruction on the H&E-stained sections by using polarised light microscopy. To our knowledge, there has been no mention of the visibility of fCCE on H&E-stained frozen polarised sections before.

BACKGROUND

Although atheromatous emboli (AE), including cholesterol crystals (CCs), fibrin, macrophages and calcification, are released into coronary circulation during percutaneous coronary intervention (PCI) in acute coronary syndrome (ACS),^{1–3} AE are divided into two main groups: classic AE and free cholesterol crystal emboli (fCCE).⁴ The number of released fCCE and their role in ACS have not been documented yet. PCI does not necessarily guarantee the recovery of postprocedure left ventricular dysfunction and relief of symptoms.⁵ Furthermore, fCCE manifesting in the coronary lumen following plaque rupture have been historically overlooked because the standard tissue preparation for light microscopy uses ethanol as a dehydrating agent that can dissolve fCCE, leaving behind empty tissue.^{5–7} In this case, we evaluated fCCE released during PCI for ACS and their relationship with myocardial injury and coronary artery obstruction on H&E-stained sections by using polarised light microscopy.⁸ To our knowledge, there has been no mention of the visibility of fCCE on H&E-stained frozen polarised sections before.^{4,5}

CASE PRESENTATION

A 68-year-old man who had respiratory distress during exercise underwent coronary angiography to treat unstable angina 23 days before death. It revealed severe stenosis in the triple vessels of

the coronary artery. PCI was performed using a Synergy stent (Abbott Vascular, Santa Clara, California, USA) in the left anterior descending artery and left circumflex artery, and a Xience V stent (Abbott Vascular), with subsequent predilation using a balloon in the right coronary artery (RCA) (figure 1). Just after PCI for grade 3 thrombolysis in myocardial infarction, he developed ventricular fibrillation and cardiopulmonary arrest; he received cardiopulmonary resuscitation, and sinus rhythm was established. Coronary angiography was performed again and detected no significant stenosis. However, CT revealed hepatic, renal and intestinal infarcts a week before the patient's death.

INVESTIGATIONS

On autopsy, the heart and other organs were excised and fixed in 10% buffered formalin. A transverse 1 cm thick slice was taken from the heart, midway between the atrioventricular valve and the apex of the ventricle. This midventricular slice included the right ventricle; interventricular septum; and anterior, lateral and posterior walls of the left ventricle. The tissue was dehydrated and embedded in paraffin wax for cutting. Sections were cut at 5 µm and stained with H&E or Masson trichrome. After 10% buffered formalin fixation, the myocardium, taken from each segment of both ventricles, was placed into a water-soluble compound (TissuMount, Chiba Medical Co., Saitama, Japan) consisting mainly of polyvinyl alcohol, sucrose, sodium alginate and glycerol. The myocardium was frozen in liquid nitrogen of −180°C, and cut 5 µm thick in the cryostat. The ribbons were unstained and H&E stained, and examined with a microscope (Olympus, Tokyo, Japan) under polarised light with a magnification of ×100–400.

The aorta had complicated lesions of American Heart Association Classification VI, and the heart weighed 610g, in which the cut surface showed multiple fibrous microinfarcts (figure 2). Additionally, the coronary arteries, except the areas of stenting, showed complicated lesions, and no significant stenosis was recognised in the coronary stents. Each segmental myocardium was traditionally examined by H&E staining without any fCCE, but polarised microscopy of H&E-stained frozen slides revealed positive polarisation of fCCE in the

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Figure 1 Final angiogram after percutaneous coronary intervention. (A) Two drug-eluting stents (distal: Synergy (2.25 mm in diameter by 32 mm in length), proximal: Synergy (2.25 mm in diameter by 16 mm in length)) were implanted in the middle part of the left circumflex artery with overlapped stenting (dashed red line). (B) A drug-eluting stent (Synergy (2.25 mm in diameter by 38 mm in length)) was implanted in the middle part of the left anterior descending artery (dashed yellow line). (C) Two drug-eluting stents (distal: Xience Alpine (2.75 mm in diameter by 28 mm in length), proximal: Xience Alpine (3.5 mm in diameter by 28 mm in length) were implanted in the proximal-to-middle part of the right coronary artery (dashed red line). (D) Two drug-eluting stents (atrioventricular branch: Xience Alpine (2.5 mm in diameter by 23 mm in length), posterior descending branch: Xience Alpine (2.5 mm in diameter by 15 mm in length)) were implanted in the distal part of the right coronary artery with culotte stenting (dashed yellow line).

capillaries and arterioles of the myocardium (figure 3A–D). The segment supplied by the RCA was relevant to many polarised crystals adjacent to the microinfarcted lesions (figure 3A–D). There were no definite inflammatory cell infiltrations or macrophages around the fCCE. Aggregated fCCE ranged between approximately 20 and 80 μ m in length. Complete occlusive fCCE were occasionally seen in the capillaries and arterioles (figure 3E,F).

OUTCOME AND FOLLOW-UP

Subsequently, complete atrioventricular block led to his death.

Laboratory data showed an eosinophil count in peripheral blood of 3.1%, estimated glomerular filtration rate of 22 mL/min, and C reactive protein level of 24.6 mg/dL, which might be due to generalised infection in the agonal period. A postmortem examination was performed.

DISCUSSION

The two main findings of the present case report are summarised as follows. First, the CCs could be observed on the slides of

Figure 2 Histopathological findings of the left ventricle. (A) Microphotogram showing multiple microinfarcts with some inflammatory cell infiltration in the posterior wall of the left ventricle detected by H&E staining and no definite cholesterol crystals in the capillaries. (B) Young fibrous tissue showing scarring detected by Masson-Trichrome staining.

H&E-stained frozen sections by polarised microscopy because the standard tissue preparation for light microscopy uses ethanol as a dehydrating agent that can also dissolve CCs, leaving behind empty tissue imprints or ‘clefts’ as seen in AE.^{8–10} As a result, when fCCE only existed on the slides, they disappeared, which is why no study has documented fCCE before. Unfortunately, the existence and significance of fCCE have been overlooked for a long time.^{11 12} As described recently,⁴ we proposed that AE were classified two main groups: AE including CCs, fibrin, red blood cells, macrophages and calcification; and fCCE including only CCs. The former will rapidly cause much more larger infarcts that are visible on imaging, such as CT, MRI and optical coherence tomography, rather than the latter; the latter will very slowly cause small or tiny infarcts that will not be recognised only on imaging studies, during the clinical course, or on histological examination.

Second, we documented small infarcts adjacent to obstructive capillaries and/or arterioles near the RCA in the left ventricle

Figure 3 H&E-stained frozen sections of the myocardial capillaries and arterioles. (A, B) Free cholesterol crystal emboli in the myocardial capillaries and arterioles shown in the frozen sections by using direct polarised light. (C, D) Free cholesterol crystal emboli (dashed blue, circular line) in the myocardial capillary on H&E-stained frozen sections along the direction of polarised light adjacent to microinfarcts (dashed red, circular line). (E, F) Complete occlusive free cholesterol crystal emboli are occasionally seen in the capillaries on H&E-stained frozen sections along the direction of polarised light adjacent to microinfarcts.

with fCCE verified by polarised microscopy. This means that the plaques led to rupture at the time of balloon dilatation before PCI, releasing a myriad of CCs into the coronary flow that protruded from between the struts of the stents. A plethora of myocardial microinfarcts might have led to complete atrioventricular block as a cause of death in this patient.

Since 1978, there have been sporadic reports of coronary artery embolism caused by AE, including CCs, fibrin and macrophages in PCI and coronary artery bypass grafting and other invasive procedures.⁸ All reports of coronary artery embolism until the present time have been determined as caused by AE, since the empty vehicles of AE are easily recognisable by regular light microscopy because of dissolution by ethanol as a dehydrating agent. According to a recent paper, aside from classic AE, fCCE were found in the peripheral blood by non-obstructive aortic endoscopy.¹³ To our knowledge, this report will be the first to mention fCCE so far, as fCCE would be dissolved by ethanol in the course of the routine processes of histopathological work.

According to Niccoli *et al.*,¹⁴ four interacting mechanisms cause coronary microvascular dysfunction and obstruction in humans: ischaemia-related injury, reperfusion-related injury, distal embolisation and individual susceptibility of microcirculation to the injury. Herein, we prefer to discuss the mechanism with regards to distal embolisation, AE of different sizes but not fCCE can originate from an epicardial coronary thrombus and from fissured atherosclerotic plaques during primary PCI; spontaneous embolisation has been also suspected before vessel manipulation.⁸ Furthermore, fCCE will be dissolved by ethanol and too difficult to recognise by a microscope on regular H&E-stained sections, and they will slowly and repeatedly produce microinfarcts anywhere in the vicinity of capillaries and/or arterioles of the heart, brain, kidneys and lower extremities.

According to Abela *et al.*,⁵ the release of CCs into the circulation has been known to induce end-organ injury and inflammation in several clinical syndromes, including amaurosis fugax, livedo reticularis, kidney injury during renal artery interventions and stroke. Myocardial injury could reasonably be added to this list, as coronary aspirates in the vast majority of patients presenting with acute myocardial infarction had CCs, with more than half of those having moderate-to-extensive amounts of crystals. We would like to think that such CCs in their report may imply classic AE, not fCCE described herein. In addition to concerns of CC emboli mentioned by those authors, the triggering of inflammation by CCs may be relevant to myocardial injury during acute myocardial infarction, and another unique finding of the study was the apparent interaction between macrophages and CCs, whereby macrophages appeared to be adherent to and eroding crystals.⁵ We reported no definite inflammatory cell or macrophage infiltration in the capillaries and arterioles with fCCE, but we could not verify the role of fCCE in triggering inflammation.¹⁵

The incidence of fCCE may be expected to rise with an increase in the ageing population and number of vascular procedures performed. The current estimates of the incidence are probably greatly underestimated because tissue preparation methods using ethanol dehydration dissolve fCCE. This could greatly skew the data in favour of a lower incidence of fCCE when using standard histological methods. New histological methods will be needed for detecting fCCE by which the small infarcts invisible on the images will be recognised on histological examinations. From

the standpoint of fCCE, an important problem will be vascular dementia due to ageing, as this might be responsible for fCCE leading to small infarcts in the brain.^{16–18}

Learning points

- ▶ The cholesterol crystals could be observed on the slides of H&E-stained frozen sections by polarised microscopy using our original method.
- ▶ Small myocardial infarcts adjacent to obstructive capillaries and/or arterioles near the coronary artery in the left ventricle with free cholesterol crystal emboli were verified by polarised microscopy.

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Competing interests KK, who is the president of Inter-tec Medicals, Co., developed non-obstructive angiography. SK is a technical consultant for Nemoto Kyorin-do Co.

Patient consent Next of kin consent obtained.

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