

Diagnosis of myocardial ischemia combining multiphase postmortem CT-angiography, histology, and postmortem biochemistry

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Abstract

Purpose The aim of this study was to assess whether the identification of pathological myocardial enhancement at multiphase postmortem computed tomography angiography was correlated with increased levels of troponin T and I in postmortem serum from femoral blood as well as morphological findings of myocardial ischemia. We further aimed to investigate whether autopsy cases characterized by increased troponin T and I concentrations as well as morphological findings of myocardial ischemia were also characterized by pathological myocardial enhancement at multiphase postmortem computed tomography angiography.

Materials and methods Two different approaches were used. In one, 40 forensic autopsy cases that had pathological enhancement of the myocardium (mean Hounsfield units ≥ 95) observed at postmortem angiography were retrospectively selected. In the second approach, 40 forensic autopsy cases that had a cause of death attributed to acute myocardial ischemia were retrospectively selected.

Results The preliminary results seem to indicate that the identification of a pathological enhancement of the myocardium at postmortem angiography is associated with the presence of increased levels of cardiac troponins in

postmortem serum and morphological findings of ischemia. Analogously, a pathological enhancement of the myocardium at postmortem angiography can be retrospectively found in the great majority of autopsy cases characterized by increased cardiac troponin levels in postmortem serum and morphological findings of myocardial ischemia.

Conclusions Multiphase postmortem computed tomography angiography is a useful tool in the postmortem setting for investigating ischemically damaged myocardium.

Keywords Postmortem angiography · Pathological myocardial enhancement · Forensic autopsy · Myocardial ischemia · Postmortem biochemistry · Troponin

Introduction

Myocardial infarction (MI) can be defined according to different perspectives related to clinical, electrocardiographic (ECG), biochemical, and pathologic characteristics. It is accepted that the term MI reflects a cardiomyocyte death caused by prolonged ischemia, the latter resulting from a perfusion-dependent imbalance between oxygen supply and demand. The rupture or erosion of a coronary atherosclerotic plaque is the most frequent underlying condition [1, 2].

Traditional assessment of patients presenting to the emergency department (ED) with suspected acute coronary syndrome (ACS), such as MI, includes clinical risk assessment based on cardiovascular risk factors with serial electrocardiograms and cardiac troponin measurements [3].

Detection of myocardial cell necrosis resulting from prolonged ischemia can be evaluated by a number of different means, including pathologic examination (contraction band necrosis, cardiomyocyte necrosis), biochemical

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marker measurement of myocardial cell necrosis in blood (cardiac troponins, MB fraction of creatine kinase), ECG recordings (ST-T segment wave and Q wave changes), and imaging modalities (echocardiography and coronary angiography). Coronary computed tomography angiography (CTA) has evolved into an alternative diagnostic testing strategy for patients with suspected ACS in the ED. Indeed, its diagnostic performance in assessing coronary stenosis has been demonstrated in numerous studies [1, 3].

In the realm of forensic pathology, the identification of MI is based on morphological findings (obtained from autopsy, histology, and immunohistochemistry) and biochemical investigation results, though the sensitivity and specificity of some techniques may differ markedly due to the onset of compositional changes [4].

Indeed, immunohistochemical methods may be influenced by several mechanisms that occur during postmortem alteration, including protein decomposition, antigen diffusion, and unspecific antibody binding in the presence of disrupted protein structures [5–8].

Troponin I and T levels have been measured in various studies in postmortem serum obtained from blood sampled at different sampling sites, such as the femoral veins, iliac veins, subclavian veins, aorta, right heart, and left heart. Femoral blood postmortem serum troponin levels have been shown to be useful in investigating the severity of myocardial damage due to various causes of death. Moreover, troponin concentrations measured after death have been demonstrated to correlate with the severity of ischemic myocardial damage, depending on postmortem intervals [4, 9].

On the other hand, additional findings have been described in recent reports. These assert that images obtained from multiphase postmortem CTA (MPMCTA) would enable not only coronary artery imaging and evaluation of luminal narrowing to be carried out, but also identification of MI areas as areas characterized by contrast enhancement within the myocardium (increased radiopacity from contrast medium, mean Hounsfield units ≥ 95). Since contrast enhancement is absent in the normal myocardium, this phenomenon is considered pathologic and is thought to be an indirect sign of a myocardial lesion [10–13].

To the best of our knowledge, no published reports exist to date having combined data obtained from MPMCTA, morphological findings and biochemical investigation results in the cases of suspected myocardial ischemia that have undergone forensic autopsies.

In the study herein described, troponin T and I concentrations were measured in postmortem serum from femoral blood in a series of forensic autopsy cases that underwent MPMCTA. Our first aim was to assess whether the identification of pathological myocardial

enhancement at MPMCTA was correlated with increased levels of these biomarkers as well as morphological findings of myocardial ischemia. Our second aim was to assess whether autopsy cases characterized by increased troponin T and I concentrations as well as macroscopic and microscopic findings of myocardial ischemia were also characterized by pathological myocardial enhancement at MPMCTA.

Materials and methods

Study design

This study was conducted during 2009–2015 and was designed as a retrospective, single center study. All cases collected for the study underwent medicolegal autopsies as requested by the inquiring authorities (the public prosecutor). MPMCTA and troponin T and I measurements were performed as part of medicolegal investigations.

MPMCTA was performed according to the standardized protocol in three phases described in a previous study [14].

Two different approaches were used. In one, 40 forensic autopsy cases (27 male subjects and 13 female subjects between 22 and 90 years of age) that underwent MPMCTA and had pathological enhancement of the myocardium (mean Hounsfield units ≥ 95) radiologically observed, were retrospectively selected. The aim was to assess whether the identification of pathological enhancement of the myocardium at MPMCTA was correlated with increased troponin T and I values as well as the morphological findings of myocardial ischemia. Death and medicolegal investigation intervals ranged between 12 and 48 h. In 10 out of 40 cases, death and medicolegal investigation intervals ranged between 36 and 48 h.

At the same time, in the second approach, 40 forensic autopsy cases (28 male subjects and 12 female subjects between 36 and 67 years of age) that underwent MPMCTA and had a cause of death attributed to acute myocardial ischemia based on macroscopic and microscopic findings as well as biochemical investigation results were retrospectively selected. The aim was to assess whether autopsy cases characterized by increased troponin T and I concentrations as well as the macroscopic and microscopic findings of myocardial ischemia were also characterized by pathological enhancement of the myocardium at MPMCTA. Death and medicolegal investigation intervals ranged between 14 and 50 h. In 13 out of 40 cases, death and medicolegal investigation intervals ranged between 34 and 50 h.

The cases included in the second case series were characterized by the presence of one of the following combinations of macroscopic and microscopic findings:

- Coronary artery atherosclerosis and acute coronary thrombosis without acute myocardial infarction.

In these cases, postmortem angiography revealed complete interruption of coronary artery opacification at various sites. Macroscopy and microscopy revealed the presence of acute thromboses in the coronary arteries as well as the presence of morphological signs of myocardial ischemia.

- Coronary artery atherosclerosis and acute coronary thrombosis with acute myocardial infarction.

In these cases, postmortem angiography revealed complete interruption of coronary artery opacification at various sites. Macroscopy and microscopy revealed the presence of acute thromboses in the coronary arteries and the presence of morphological signs of myocardial infarction.

- Rupture or erosion of a coronary atherosclerotic plaque, with or without hemorrhage within the plaque, with or without acute myocardial infarction.

In these cases, postmortem angiography revealed various degrees of coronary artery atherosclerosis with no evidence of significant luminal narrowing. Microscopy revealed the presence of rupture or erosion of a coronary atherosclerotic plaque, with or without hemorrhage within the plaque and with or without morphological signs of acute myocardial infarction.

- Acute myocardial infarction with or without rupture left ventricular free wall rupture.

In these cases, postmortem angiography revealed various degrees of coronary artery atherosclerosis, with or without evidence of interruption of coronary artery opacification. Macroscopy and microscopy revealed the presence of myocardial infarction, with or without left ventricular free wall rupture, with or without acute thromboses in the coronary arteries.

- Coronary artery atherosclerosis, without acute coronary thrombosis, with morphological signs of myocardial ischemia.

In these cases, postmortem angiography revealed various degrees of coronary artery atherosclerosis without evidence of interruption of coronary artery opacification. Macroscopy and microscopy failed to reveal the presence of acute thromboses in the coronary arteries and revealed the presence of morphological signs of myocardial ischemia.

All cases selected for this study originated from forensic practice with deaths occurring outside the hospital. All cases underwent medicolegal autopsies as requested by local inquiring authorities. MPMCTA and biochemical investigations were performed as part of medicolegal investigations. Medical records and clinical histories of the deceased as well as police reports were consistently reviewed before conclusions were made.

Case inclusion criteria for the first studied case series consisted of pathological myocardial enhancement at MPMCTA and postmortem serum from femoral blood availability at autopsy.

Case inclusion criteria for the second investigated case series consisted of morphological signs of acute myocardial ischemia or myocardial infarction and postmortem serum from femoral blood availability at autopsy.

Postmortem investigations and sample collection

Unenhanced postmortem computed tomography (CT) scans were performed before any manipulation of the corpses in all cases included in the study. MPMCTA was systematically carried out after unenhanced CT scans and prior to autopsies, according to the standardized protocol in three phases described in a previous study [14]. Complete, conventional medicolegal autopsies, histology, and biochemical investigations were performed in all cases. Autopsies were jointly performed by two forensic pathologists (at least one board-certified) as in accordance with both local standards and international guidelines for medicolegal cases.

The presence or absence of pathological myocardial enhancement and its distribution within the myocardium was investigated by at least one board-certified forensic pathologist (experienced in forensic imaging) together with at least one board-certified radiologist unbeknownst to the results of previous investigations. The pathological enhancement of the myocardium was first identified in a subjective manner (the presence or absence of enhancement according to the observers) and second in an objective manner, by measuring the mean attenuation (in Hounsfield units) of the myocardium (using a region of interest of about 15 mm in diameter) in the images of the three different MPMCTA phases.

Conventional histology included haematoxylin–eosin (HE) stains of brain, heart, lung, liver, and kidney samples. HE staining was performed after tissue fixation in formaldehyde. Hearts were sectioned before or after fixation in 10 % neutral buffered formalin. The major epicardial coronary arteries were either serially sectioned at approximately 2-mm intervals or longitudinally sectioned intact on the heart. In selected cases, histological sections of the coronary arteries were prepared at three different equally

spaced levels to best identify plaque rupture sites. Full thickness areas involving the left anterior, lateral free wall, and left posterior ventricle as well as interventricular septum, and the right anterior, lateral free wall and right posterior ventricle were sampled. Affected coronary arteries were removed from the heart and were retained for decalcification prior to dissection. In selected cases, histology staining analysis for coronary arteries included HE, Masson's trichrome and Verhoeff van Gieson as well as immunohistochemical investigations.

Toxicological analysis was performed in selected cases and included blood ethanol determination as well as general screening for volatile and nonvolatile drugs, poisons, and metabolites.

Peripheral blood from femoral veins was systematically collected for postmortem biochemistry as soon as possible upon arrival of the bodies at the morgue, prior to autopsy and prior to MPMCTA. Femoral blood samples were collected by aspiration with sterile needles and syringes from the femoral vein(s). Blood samples were drawn after clamping the vein(s) at the proximal end and keeping the lower limb(s) raised for several minutes. Samples were stored in tubes containing sodium fluoride and preservative-free gel serum separator tubes. The latter were centrifuged immediately post collection at 3000g for 15 min. After centrifugation, the separated supernatant (postmortem serum) was collected and stored in preservative-free tubes. No specimens were excluded due to insufficient sample volume. Postmortem serum samples were transferred to the laboratories immediately post collection. When analyses were delayed, samples were stored at -20°C .

Technical data

Chemically, the contrast medium AngiofilTM is a mixture of esters (mainly ethyl esters) of polyiodinated fatty acids. This means that it is an oily liquid with all the chemical properties known and inherent to such compounds. It is yellowish, nearly odorless and stable at room temperature. The advantages of using oily liquids for injection into vessels are well known and have been described in the literature. One study stated that combining an oily perfusate with a lipophilic contrast medium allows postmortem circulation and the performance of subsequent high-resolution postmortem angiography to be carried out. Microscopic studies reported in this same paper demonstrated that oil induces fatty micro-embolisms in capillary circulation and enters the venous system via small arteriovenous shunts. This, therefore, arrests capillary microcirculation, much more permeable after death due to decompositional changes, rendering oily perfusates highly suitable for postmortem angiography. The same mechanism is used in cancer treatment to split tumors off from their blood supply during

chemoembolization. Since the level of micro-embolisation depends on the viscosity of the oily perfusion, the use of an adequate perfusion liquid is of paramount importance. If the contrast agent AngiofilTM is dissolved in paraffin oil, its viscosity will be the same as that of the oil used. Paraffinum liquidum has appeared the most appropriated with which to perfuse a human body, while the use of the more viscous paraffinum perliquidum can lead to important extravasations, especially in areas where postmortem autolysis is more significant, such as the pancreas and gastric mucosa. By diluting AngiofilTM with a solvent such as decane, viscosity can be decreased to the extent that it can enter the capillaries and, therefore, be used as a contrast medium in micro-angiography [8]. Using AngiofilTM together with paraffinum liquidum as a solvent, as proposed per the standardized protocol of multiphase PMCTA, the kinematic viscosity of the mixture measured at 40°C is approximately $67\text{--}74\text{ mm}^2/\text{s}$ [10, 14].

Histological alterations induced by oily contrast agents

Using oily contrast agent has the advantage that no leakage out of the vascular system occurs within the first 72 h. Another advantage is the absence of diffusion into the surrounding tissue. With water-soluble contrast agents, diffusion into the tissue depends on the concentration. In the cases of advanced decomposition, contrast agent-mediated oedema often appears, even with the use of an oily contrast agent. The stomach, gut, and pancreas are particularly affected by these contrast enhancements. Optical empty spaces within the vessels or even within the parenchyma are among the most common histological artifact after MPMCTA with oily contrast agent and are the histological analog to radiologic contrast agent-mediated oedema. Optical empty spaces frequently appear in all tissues [10].

Laboratory assays

Cardiac troponin I was analyzed with the Access[®] AccuTnITM assay on Access II (Beckman Coulter, Fullerton, CA, USA). Results were expressed in $\mu\text{g/l}$. The clinical reference value (according to the laboratory where the analysis was performed) was $0.03\text{ }\mu\text{g/l}$ (corresponding to 0.03 ng/ml).

Levels of postmortem serum cardiac troponin T were measured with *hs*-TnT reagents by electrochemiluminescence immunoassay (ECLIA). Results were expressed in ng/l . The clinical reference value (according to the laboratory where the analysis was performed) was 14 ng/l .

Toxicology consisted of ethanol determination and general unknown screening for common drugs and illegal substances by gas chromatography-mass spectrometry (GC-MS) using commercial mass spectrum libraries,

high-performance liquid chromatography with diode-array detection (HPLC–DAD), and headspace-gas chromatography flame ionization detection (HS–GC–FID).

Ethics

All relevant ethical issues were identified and discussed with the local Ethical Committee. All cases collected for this study underwent medico-legal autopsies as requested by the public prosecutor. Postmortem serum from femoral blood is systematically collected in our facility prior to or during autopsy. Moreover, MPMCTA and biochemical investigations are routinely performed. All data were anonymized prior to analysis. No further ethical approval was necessary to perform MPMCTA and biochemical investigations in the cases included in this study.

Results

Main results are summarized in Table 1. Figure 1 summarizes the main radiological, macroscopic and microscopic findings in a case of pathological myocardial enhancement at MPMCTA correlated with morphological findings of myocardial ischemia. All the presented images pertain to the same case in which pathological myocardial enhancement and morphological findings of myocardial ischemia were identified.

In the first case series (40 cases), the identification of pathological myocardial enhancement (mean Hounsfield units ≥ 95) at MPMCTA systematically correlated with increased levels of troponin T and I as well as morphological findings of myocardial ischemia. The cause of death in this series was considered to be acute myocardial ischemia or acute myocardial infarction in 37 out of 40 cases.

In these 37 cases, postmortem serum troponin concentrations were systematically increased and ranged (troponin I) from 1.21 to 10.16 $\mu\text{g/l}$ (mean value 4.67 $\mu\text{g/l}$) and (troponin T) from 32 to 98 ng/l (mean value 58 ng/l). No statistically significant differences between troponin concentrations and postmortem interval (12–36 and 36–48 h) were observed.

No case was noticed that had pathological myocardial enhancement at MPMCTA without increased troponin levels or without morphological findings of myocardial ischemia. No case had exclusively increased troponin levels without morphological findings of myocardial ischemia, irrespective of the postmortem interval.

Analogously, no case had exclusively morphological findings of myocardial ischemia without increased troponin levels, irrespective of the postmortem interval.

The enhancement of the myocardium at MPMCTA correlated with the localization of the ischemia

(macroscopically and microscopically identified) in all 37 cases. In some of these cases, the enhancement of the myocardium at MPMCTA was markedly pronounced (>200 Hounsfield units) in the subendocardial areas of the left ventricle, where former infarcted areas were also histologically noticed. While the arterial phase of the MPMCTA showed diffuse myocardial enhancement in most cases, this was more pronounced in the subendocardial regions of the myocardium during the venous and dynamic phases of the MPMCTA.

In the remaining 3 out of 40 cases, pathological myocardial enhancement at MPMCTA correlated with increased levels of troponin T and I as well as various degrees of myocardial ischemia. The causes of death were considered to be septic shock, pulmonary embolism and trauma following a fall from heights in a subject with severe coronary artery atherosclerosis, coronary artery calcifications, and myocardial fibrosis. Postmortem serum troponin concentrations were increased in all three cases (troponin I 1.21, 1.89, and 2.13 $\mu\text{g/l}$, respectively, and troponin T 29, 36, and 43 ng/l, respectively).

It is not surprising that increased levels of cardiac troponins as well as morphological findings of myocardial ischemia might be found in the cases of severe sepsis and septic shock. Elevated cardiac troponin levels in sepsis have been shown to occur in the absence of coronary thrombosis. The reasons for the increase in troponin concentrations are not clearly delineated, though several hypotheses have been formulated. These include myocardial ischemia and direct myocardial damage due to substances released into the circulation by pathogens, cytokines, or reactive oxygen radicals released following the infectious process [15–17].

In pulmonary embolism, the sudden elevation of right ventricular pressure, and consequently increased right ventricular afterload produced by pulmonary artery outflow obstruction, results in right ventricular failure and dilatation. This induces myocardial ischemia, responsible for cardiac troponin elevation in blood. Moreover, the sudden increase in pressure on the right ventricle results in myocardial cell stretching and acute coronary vasospasm, both situations worsening myocardial ischemia. Finally, the severe hypoxemia that accompanies massive pulmonary embolism induces a catecholamine surge and further increases myocardial workload, thus aggravating the ischemia [18].

In the second case series (40 cases), increased troponin T and I levels as well as the morphological findings of myocardial ischemia were correlated with pathological myocardial enhancement (mean Hounsfield units ≥ 95) at MPMCTA in 38 out of 40 cases.

Postmortem serum troponin concentrations were increased in all cases included in this case series and ranged (troponin I) from 1.11 to 8.87 $\mu\text{g/l}$ (mean value 3.64 $\mu\text{g/l}$) and (troponin T) from 39 to 96 ng/l (mean value

Table 1 Summarizes the main results pertaining to the studied groups

	CT values of pathological enhancement of the myocardium (Hounsfield units)	Troponin T concentrations (range) (ng/ml)	Troponin I concentrations (range) ($\mu\text{g/l}$)	Pathological findings at autopsy/histology (myocardium/coronary arteries)	Postmortem intervals (h)	Identified cases
Pathological enhancement cases (40 cases)	96–200	32–98	1.21–10.16	Myocyte hyper eosinophilia Contraction band necrosis Hemorrhage Myocyte necrosis Neutrophilic inflammatory infiltrate Occlusive coronary thrombosis Plaque complications (rupture/hemorrhage)	12–48	Pathological enhancement of the myocardium systematically correlated with morphological findings of myocardial ischemia and increased troponin levels In 3 out of 40 cases, the cause of death was not considered to be myocardial ischemia
Myocardial ischemia cases (40 cases)	96–200	39–96	1.11–8.87	Myocyte hyper eosinophilia Contraction band necrosis Hemorrhage Myocyte necrosis Neutrophilic inflammatory infiltrate Occlusive coronary thrombosis Plaque complications (rupture/hemorrhage)	14–50	38 out of 40 cases: morphological findings of myocardial ischemia or myocardial infarction and increased troponin levels correlated with pathological enhancement of the myocardium 2 false negative cases at MPMCTA

61 ng/l). No statistically significant differences between troponin concentrations and postmortem interval (14–34 and 34–50 h) were observed.

No case had exclusively increased troponin levels without morphological findings of myocardial ischemia, irrespective of the postmortem interval. Analogously, no case had exclusively morphological findings of myocardial ischemia without increased troponin levels, irrespective of the postmortem interval.

As revealed in the first case series, myocardial enhancement at MPMCTA correlated with the localization of the narrowing of the coronary artery and the localization of the myocardial ischemia (macroscopically and microscopically identified) in 38 subjects. Most revealed diffuse enhancement of the myocardium in the arterial phase of the MPMCTA in most cases and more pronounced enhancement in the subendocardial regions of the myocardium during the venous and dynamic phases.

In the remaining two cases, increased levels of troponin T and I (troponin I 0.62 and 0.89 $\mu\text{g/l}$, troponin T and 33 ng/l) and morphological findings of myocardial ischemia did not correlate with pathological myocardial enhancement. This could be due to the fact that infarcted areas of very limited extension might not be detectable at MPMCTA as pathological enhancement.

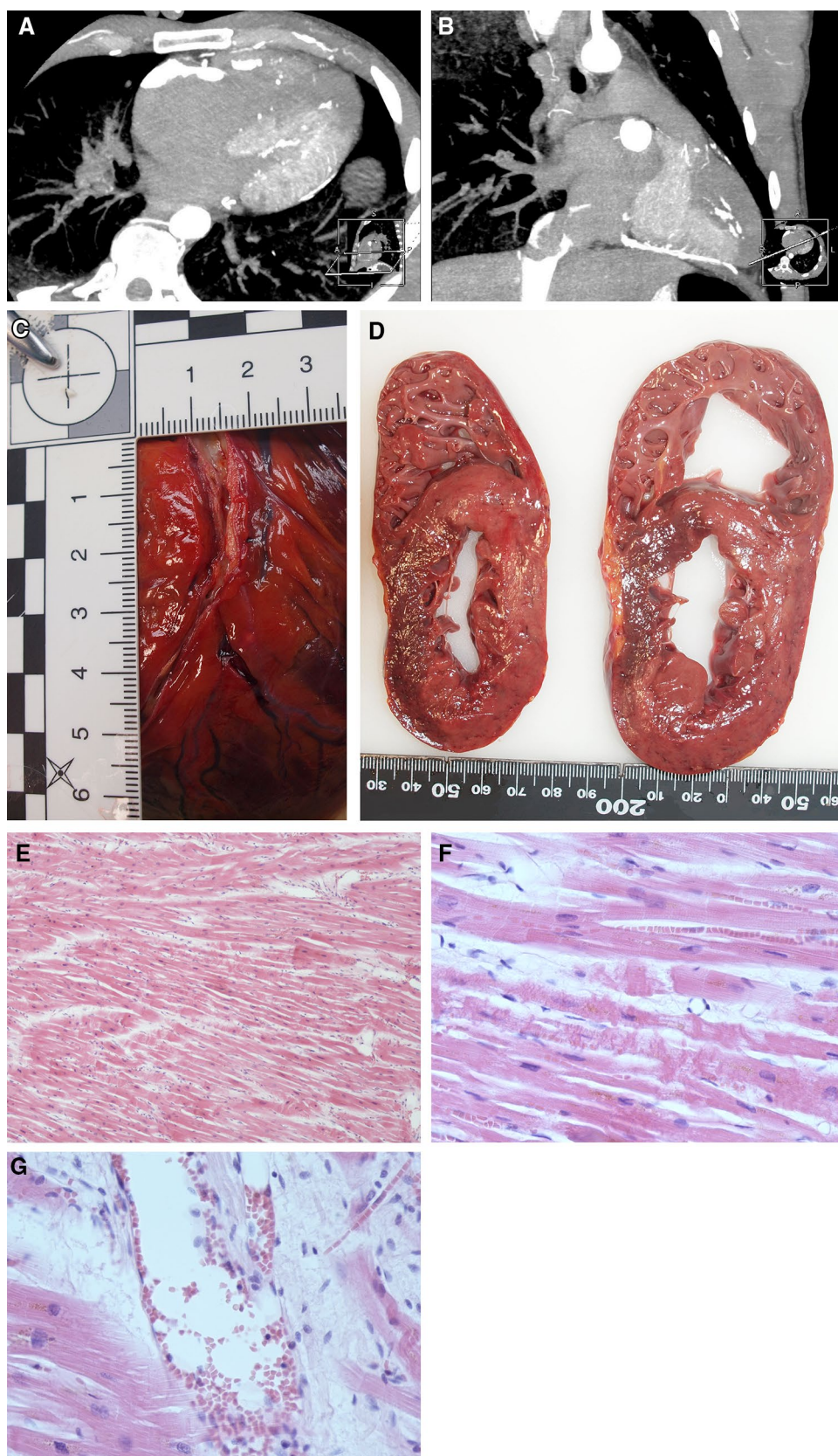
Globally considered, these results indicate that the identification of a pathological enhancement of the myocardium at MPMCTA (mean Hounsfield units ≥ 95) is strongly associated with the presence of increased levels of cardiac troponins in postmortem serum and morphological findings of ischemia, irrespective of whether myocardial ischemia is considered the main cause of death, a factor contributing to death or just a potential trigger factor, as hypothesized in the case of the fall from heights in a subject with severe coronary artery atherosclerosis.

Analogously, a pathological enhancement of the myocardium at MPMCTA can be retrospectively found in the great majority of autopsy cases characterized by increased cardiac troponin levels in postmortem serum and morphological findings of myocardial ischemia. This suggests the usefulness of combining MPMCTA and postmortem biochemical analyses for the investigation of ischemically damaged myocardium.

Discussion

Ischemic injury of the myocardium can be differentiated as reversible and irreversible, as well as present in acute and chronic conditions. Myocardial stunning and hibernating myocardium are examples of reversible acute and reversible chronic situations, respectively, in which the myocardium is hypocontractile but viable. Myocardial stunning

Fig. 1 The main radiological, macroscopic, and microscopic findings in a case of pathological myocardial enhancement at MPMCTA correlated with morphological findings of myocardial ischemia. All the presented images pertain to the same case. **a, b** The contrast enhancement within the myocardium (increased radio-opacity from contrast medium) identified at MPMCTA in a situation of narrowing of the coronary artery and myocardial ischemia. **c** The narrowing of the left descending coronary artery in the same case illustrated in **a, b** and well as in **d–g**. **d** The macroscopic aspect of the myocardium in the same case. *Brown areas* can be noticed within the left anterior free wall and the interventricular septum. **e** (Haematoxylin–eosin 10 $\times$) and **f** (haematoxylin–eosin 40 $\times$) show the microscopic aspect of the myocardium in the same case. Histological sections were obtained from the left anterior free wall and corresponded to the brown areas macroscopically identified (**d**). Contraction band necrosis can be microscopically observed. **g** Optical empty spaces within the vessels induced by the oily contrast material



refers to myocardium that is salvaged by timely reperfusion with transiently impaired contractile function that recovers spontaneously over time. Hibernating myocardium refers to myocardium with chronically reduced contractile function associated with a local reduction in myocardial perfusion capacity [19, 20].

Ischemic cardiomyocyte injury is characterized by progressive membrane damage with a variable degree of cell swelling. Ischemic cell membrane injury has been shown to proceed from discrete alterations in specific membrane pumps and ion channels to an intermediate stage. This stage is less selective with increasing membrane permeability and, consequently, more severe ionic disturbances. These may include increased calcium ion influx to a final stage of membrane rupture. This loss of cell membrane integrity marks the point of cell necrosis and irreversible myocardial infarction. Not all cells die simultaneously in acute myocardial infarction. Instead, myocyte necrosis starts in the subendocardial layers of the myocardium where energy demand is greatest. With prolonged ischemia, myocyte death spreads like a wave across the myocardium. The extent of necrosis depends on the occluded vessel's territory, which also determined ischemia duration and the transmural extent of the infarction. The necrotic myocardium is replaced by scar tissue within 6 weeks. Scar tissue is markedly thinner than healthy myocardium. The time course of this remodeling process is influenced by several factors and encompasses underlying disease or secondary event severity [19–25].

While the clinical use of differential contrast enhancement of ischemic myocardium is a relatively recent advance, the concept of differential enhancement/accumulation of contrast material in acute or chronic myocardial infarction in both cardiac magnetic resonance imaging (MRI) and computed tomography (CT) imaging dates back to studies published in the late 1970s [26].

In the initial studies that investigated contrast material uptake by ischemically damaged and normal myocardium, iodinated contrast media and CT of extirpated canine hearts with acute and chronic myocardial infarctions were used. Delayed scans obtained within minutes of contrast material administration revealed iodine concentrations of infarcted tissue several times greater than that of normal myocardium, irrespective of the injected contrast material (meglumine/sodium diatrizoate, iodipamide, and an experimental polymer of iothalamic acid). Postmortem histological studies including histochemical morphometry confirmed that the region of enhancement closely corresponded to the spatial extent of infarcted myocardium, including healed scars. The enhancement of acute infarctions was later shown in the in situ beating heart using a prototype electrocardiography (retrospective)-gated CT scanner in the early 1980s [26–36].

Great advances have been made in postmortem imaging during the last decade and postmortem CTA has become a field of intense research. Different approaches of investigating the whole body or coronary arteries only have been developed in numerous medicolegal centers. Even though proposed protocols differ as per injection method or contrast material, they all have one point in common. They have all been demonstrated to significantly increase the quality of postmortem investigations, especially with regard to vascular system injury and disease [10, 37].

Specifically, concerning the differential enhancement/accumulation of contrast material in ischemically damaged and normal myocardium, recent reports have indicated that MPMCTA (using the standardized protocol in three phases and a lipophilic contrast agent mixture) would allow infarcted regions of the myocardium to be identified as areas characterized by pathological enhancement. This would likely be due to an abnormal leak in the infarcted myocardium. In comparison, normal myocardium does not reveal any contrast material accumulation [10–13].

As cardiac myocytes become necrotic, intracellular proteins [myoglobin, lactate dehydrogenase, MB fraction of creatine kinase (CK-MB), troponins] leak into the interstitial space and enter the systemic circulation via local microvascular and lymphatic drainage. The concentration–time profile for these markers in peripheral blood depends on their molecular weight, and location within the myocytes as well as their rates of vascular or lymphatic drainage and systemic clearance. Small cytosolic proteins, such as myoglobin, are detectable within 1–2 h after tissue injury, whereas large enzymes such as lactate dehydrogenase diffuse much more slowly [38].

Troponin complex is a component of skeletal and cardiac muscle thin filaments. It consists of three subunits: the calcium binding sub-unit, troponin C (TnC); the inhibitory sub-unit, troponin I (TnI); and an elongated sub-unit, troponin T (TnT), that binds both TnC and TnI, anchoring the entire complex to tropomyosin [39].

Troponins play a crucial role in muscle activity, connecting changes in intracellular Ca^{2+} concentrations with contraction generation. TnI is expressed in three isoforms. In humans: fast and slow skeletal isoforms and specific cardiac isoform. TnT is expressed in humans by three genes coding slow and fast skeletal and cardiac isoforms of the protein. TnC is expressed in humans by two genes: one located on chromosome 3 that codes the cardiac/slow skeletal isoform of TnC and one located on chromosome 20 that codes the fast skeletal isoform of TnC. TnT plays the main role in troponin complex fixation on the actin filament, organizes the subunits in the complex, and participates in muscle contraction regulation. In an adult human heart muscle, the 35.9-kDa isoform TnT3 is predominantly expressed [40].

Cardiac troponin C (cTnC) contains two high affinity calcium binding sites that are always occupied by Ca^{2+} or Mg^{2+} under physiologic conditions, stabilizing an open conformation that remains anchored to the rest of the troponin complex. During muscle activation, calcium binding to cTnC favors an open conformation that binds to the switch region of cTnI, removing adjacent inhibitory regions of TnI from actin and allowing muscle contraction to proceed. TnI is the inhibitory sub-unit of the troponin complex associated with the thin filament. It inhibits actomyosin interactions at diastolic levels of intracellular Ca^{2+} [39, 40].

Cardiac troponins are coded by specific genes and theoretically have the potential of being unique to the myocardium. Indeed, cardiac troponin I (cTnI) has not been identified outside the myocardium. Cardiac troponin T (cTnT) is expressed to a small extent in skeletal muscle; however, current cTnT assay does not identify skeletal troponins. Cardiac troponins are detected in the serum by the use of monoclonal antibodies to epitopes of cTnI and cTnT. These antibodies are highly specific for cardiac troponin and have negligible cross reactivity with skeletal muscle troponins. cTn-T and cTn-I are highly sensitive, specific markers of myocardial necrosis in patients with acute myocardial infarction. They may not be detected in the serum for up to 4 h after an acute coronary event's onset. Their concentrations remain raised for four times longer than CK-MB concentrations because of the sustained release of structurally bound protein from disintegrating myofibrils [38–43].

cTn-T and cTn-I are measured in routine clinical investigations of myocardial damage, mainly for the diagnosis and management of myocardial infarction. The use of cardiac troponins as a part of laboratory analyses in forensic autopsy has been investigated by several research teams. Numerous reports have suggested the possible applications of these markers for the evaluation of the presence and extent of myocardial damage in various causes of death. Postmortem cardiac troponin levels depend on numerous factors, including the severity and duration of myocardial damage before death, cause of death, postmortem interval and sampling site. Hence, differences in clinical findings should be carefully considered when evaluating postmortem data and clinical reference values should not be directly used in interpreting results obtained from postmortem serum samples. These limits notwithstanding, femoral blood postmortem serum troponin levels have been shown to be reliable in investigating the severity of myocardial damage and correlate with the ischemic myocardial damage severity [4, 9, 44–48].

The results of the study presented herein support the hypothesis that pathological enhancement of the myocardium at MPMCTA (mean Hounsfield units ≥ 95) combined with further postmortem analysis results, such as troponin I

and T determination, may be useful in the investigation of myocardial ischemia and myocardial infarction.

This is the first study, to the best of our knowledge, to assess the radiological profile of myocardial ischemia combined with further postmortem investigation results (with specific regard to cardiac troponins) in a series of cases that had undergone forensic investigations, including postmortem angiography, histology, and biochemistry. We were unable to find similar studies in the forensic setting with which to compare our results.

Our present study has some limitations. The most important is the relatively small number of studied cases, which may limit the accuracy of our research. However, precise selection criteria were applied during the recruitment process in all study groups and subgroups to minimize heterogeneity in the study populations. Prospective investigations including a greater number of subjects are, therefore, needed to confirm our findings.

In conclusion, though additional studies are required to confirm these preliminary observations, our results indicate that MPMCTA is a useful tool in the postmortem setting, not only for characterizing coronary artery morphology and detecting coronary artery stenosis, but also for investigating ischemically damaged myocardium. This is especially true when the presence of pathological myocardial enhancement is combined with further postmortem investigation results, such as increased troponin levels.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards This article does not contain any studies with human participants or animals performed by any of the authors.

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