Observational Study

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Early Postmortem Interval Estimation in Forensic Radiology: A Proof-of-Concept Study Using qMRMI and Tractography in a Porcine Heart Model

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Abstract

Estimating postmortem interval (PMI) is a difficult task in daily forensic practice, because of all the limitations of most of the methods used for its determination. Over the years, numerous attempts have been conducted to overcome these limitations. Among these, the improvement and innovation of postmortem radiological techniques may play an important role. In this preliminary study, a novel approach for early postmortem interval estimation (ePMI) using quantitative magnetic resonance molecular imaging (qMRMI) is proposed. The study made use of a pig heart, whose anatomical and structural characteristics are known to be like those of humans. The sample was obtained from the commercial food chain, having been taken immediately after the animal was slaughtered. The pig heart was maintained at 17 °C degree. Radiological investigations have been conducted using a 1.5 T MRI scanner, and 2 scans have been performed. Fractional anisotropy (FA), tractography and susceptibility weighted changes due to the heart degradation processes have been monitored. The image acquisition was performed at 3.5 h after death (T0) and 24 hours after death (T1). Results showed measurable time-dependent variations, with FA and AD decreasing and ADC and RD increasing, because of the progressive disorganization of normal myocardiocytes architecture in the postmortem era. Although limited to a single case and two time points, this exploratory study suggests that postmortem qMRMI could be a potentially powerful tool for early PMI estimation. In the future, further studies with larger sample sizes and multiple intervals are needed to validate these preliminary observations. ***Clin Ter 2026; 177 (2):272-278 doi: 10.7417/CT.2026.2005?***

Keywords: *Forensic radiology; postmortem protein degradation; pig heart; qMRMI; PMI estimation; postmortem imaging; PMMRI*

Introduction

PMI represents the time interval occurring between the death of an individual and its body discovery (1). Although trying to correctly estimate the PMI is of fundamental importance in carrying out the activities of the forensic pathologists, currently there are no methods that allow its exact determination (2). In fact, nowadays, it is only possible to identify relatively narrow time periods within which to place the time of death, and many times this estimate is not

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sufficient to satisfy real needs (1). To estimate the time of death, three widely recognized methods are mainly used: the measurement of rectal and body temperature using the Henssge nomogram, the observation of cadaveric lividity and the evaluation of rigor mortis.

Despite this, as it is known, the effectiveness of the aforementioned evaluation tools in estimating the PMI is limited by both intrinsic and extrinsic confounding factors, capable of influencing their reliability, especially in cases of PMIs exceeding the 24 hours (3). These problems became evident early on of these techniques' application and so required the introduction of some correction factors (3). To attempt to overcome these problems, it was necessary to adopt two different Henssge nomograms based on the environmental temperature, with a cut-off value of 23°C (4). Despite this, it is known that the nomogram is applicable exclusively within 24 to 48 hours after death, with an error variability that can even exceed 7 hours (4).

Given the need to reduce as much as possible the time interval within which to collar the time of death, in recent years the scientific community has tried to develop new methods that allow these limits to be overcome, with a consequent significant increase of forensic research. In fact, to date, the relevant literature offers studies on the use of forensic entomology, the variation of the microbiome, and on the analysis of the degradation of proteins, DNA and RNA, to try to reduce the time interval within which to collar the time of death (5-9).

In the last decades, the improvement of molecular techniques, (e.g. Western Blot, Real Time Polymerase Chain Reaction) useful for studying the degradation of nucleic acids and proteins, has allowed to evaluate new aspects of those methods used for estimating the PMI (10,11). The scientific community has paid increasing attention to the study of DNA and RNA degradation processes, adopting approaches such as the quantitative amplification of specific target genes or the analysis of the integrity of nucleic acids by means of degradation profiles obtained with electrophoretic techniques. It was possible to identify a progressive time-related degradation of DNA and RNA molecules, which however remain influenced by numerous factors, such as temperature variations: the degradation of DNA within same tissues occurs more slowly at low temperatures, while are usually faster at higher temperature (10). Different degradation time of the same molecules have also been documented in different tissues, highlighting as the tissue itself can determine variation in the time degradation of these molecules, independently from temperature (10). Comparable outcomes have been obtained by evaluating RNA molecules: it seems that its degradation speed is directly proportional to the body mass index (BMI), rising in parallel with body mass index itself (11).

Therefore, despite some limitations, it should be highlighted that the use of these methods, especially if integrated with the previous ones, could be promising to reach a more precise estimation of the PMI (10). Radiological examinations represent another widely used tool in forensic pathology to assess postmortem early changes in both bone and parenchymal structures, such as the presence of intra-osseous and intra-hepatic gas, gaining increasing importance in achieving even more refined diagnoses (11,13).

Nowadays, postmortem computed tomography (PMCT) is the most widely used method, as it is not burdened by high costs, and it is extremely rapid in acquiring images (13,14). Furthermore, both the image acquisition and the analysis capacity are not affected by temperature variations (13,14-16).

Another radiological method, widely used in the clinical field, with high potential applications in forensic medicine, is represented by nuclear magnetic resonance (NMR), although this imaging technique requires significantly longer image acquisition times and are burdened by higher costs (17). For a long period of time, forensic medicine has made use of post mortem NMR (PMMR) exploiting the acquisition of conventional "morphological" images, and, therefore, using T1 - T2 weighted sequences and the short-tau inversion recovery (STIR) ones: in this case, the T1 and T2 tissue relaxation times and the proton density (PD) are exploited, leading to the formation of the so-called "magnitude images". The characteristic of these images is that they only allow a qualitative evaluation, which are well-known to be operator-dependent (18,19).

To overcome these problems, the use of quantitative methods, such as qMRMI is increasingly widespread (19,20). The formation of images in qMRMI depends on the different PD, T1, T2 relaxation times, with acquisition of numerical data, which are objective and, therefore, not burdened by subjective evaluations by the operator. These images are based (i) on the study of the isotropic and anisotropic diffusion of water molecules in the interstitial spaces, obtained with Diffusion Weighted Imaging (DWI) and FA; (ii) on the quantitative evaluation of any air present in the tissues - secondary to the onset of putrefactive phenomena- through the use of Susceptibility Weighted Imaging (SWI); (iii) on the evaluation of macromolecular structures, through the use of Magnetization Transfer (MT) 19,20).

Recently, a study by Sapienza et al. focused on the possibility of performing a more precise ePMI estimation through the analysis of the striated skeletal muscle postmortem degradation, conducted with qMRMI (12). In forensics, early PMI commonly refers to the first 24 hours after death (12). This time interval is of extreme importance in forensic settings, and therefore methods that allow an increasingly accurate estimate are considered of fundamental importance (7,8).

The aim of this article is to evaluate the effectiveness of quantitative molecular imaging techniques in the evaluation of morphostructurals postmortem transformation of myocardial fibers, and so to better define the so-called ePMI.

Materials and Methods

A pig heart was used as a model, obtained from an animal slaughtered according to standard procedures for the food chain under the supervision of a veterinary surgeon. The pig was a "*Sus Scrofa Domesticus"*, large white breed, 10 months old, weighing 100 kilograms, male, and in good health (Figure 1). This model was chosen due to the morphostructural characteristics of the pig heart, which closely resemble those of the human heart. Furthermore, the postmortem alteration phenomena occurring in the pig heart are

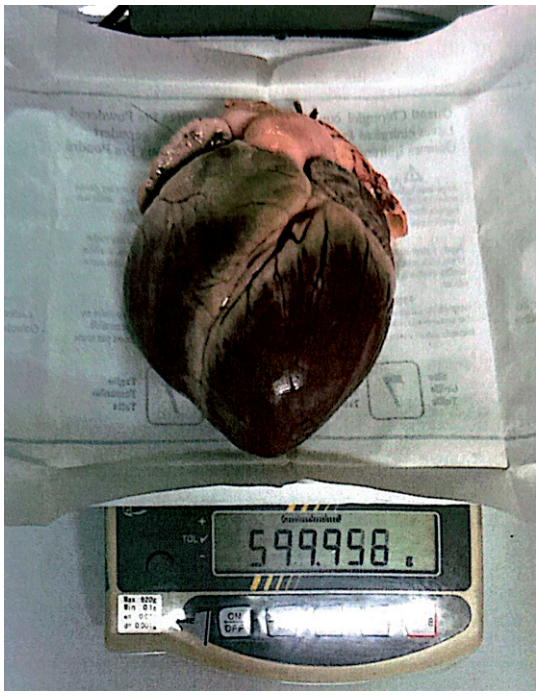

Figure 1. Isolated pig heart used as a model for postmortem radiological evaluation.

similar to those observed in humans. No authorization from the Ethics Committee was necessary because the sample was obtained from the commercial food chain.

The exact time of the pig's death was recorded. The pig heart was immediately removed and kept at a constant temperature of 17°C in a suitable and dedicated environment, with 40% humidity. The heart' right and left chambers were filled with gel to allow a better visualization of its morphology during the scans. The heart weight after the gel administration was 600 grams. The first MRI scan was carried out 3.5 hours after the time of death/collection, and it was defined as "T0". The second one at 24 hours after death, being defined as T1.

Before each acquisition, both the environmental and the heart temperature were measured (always at auricle), making use of a CE TP101 digital device with a pointed probe, with a temperature range of -50° C to +300° C, in order to track postmortem cooling. A control measurement was also performed at 10 hours after collection.

Magnetic resonance imaging acquisitions were performed using a 1.5 Tesla scanner (Ingenia, Philips, Netherlands), equipped with a 16-channel full-body coil, capable of ensuring high image quality and complete anatomical coverage. The imaging protocol included the collection of several quantitative parameters, including isotropic and anisotropic scattering measurements, useful for investigating the microstructural organization of tissues, the MT ratio, indicative of the interactions between molecular compartments, and tissue magnetic susceptibility, used to evaluate the local magnetic properties of biological tissues (21–23).

The MRI exam protocol includes:

- T1-Weighted Spin Echo Sequence: TR 684 ms, TE 10 ms, NSA 1, 308x183 matrix, scan duration: 2m 07s. These images were used as for anatomical assessment;
- Diffusion Tensor Imaging (DTI): TR 3979 ms, TE 8 ms, matrix 160x128, SL 2.2mm, directional resolution 32 scan duration 8m53s.

The data processing was performed with a dedicated console (IntelliSpaceTM 7.0) and shared by radiologists and forensic pathologists, with the purpose of evaluating the quantitative postmortem transformations of MR molecular imaging.

From the analysis of the magnetic resonance sequences of each performed scan, the following molecular imaging values were obtained:

1. Apparent Diffusion Coefficient (ADC) from DWI, automatically calculated by the machine and measured via Region Of Interest (ROI), visualized as a parametric map that reflects the degree of diffusion of water molecules through the tissue.
2. The Fractional Anisotropy from DTI, automatically calculated by the machine and measured via ROI, expresses the degree of directionality of the movement of the molecules and therefore gives information on the architecture of the fiber bundle.
3. Axial diffusivity (AD), denoted as $\lambda \parallel \equiv \lambda_1$, represents the average diffusion coefficient of water molecules moving parallel to the main direction of the tract within the analyzed voxel. This parameter reflects the diffusion along the longitudinal axis of the fibrous structures.
4. Radial diffusivity (RD), defined as $\lambda^{\perp} \equiv (\lambda_2 + \lambda_3)/2$, instead quantifies the average diffusion of water molecules in a direction perpendicular to the tract.

The molecular imaging values were obtained by placing ROI within the basal and apical septum and spread across all the acquired sequences. Tractography was obtained using the Philips complementary console processing program.

Results

Time-related variations of fractional anisotropy, apparent diffusion coefficient, axial diffusivity and radial diffusivity

It was observed the variation of the FA, ADC, AD and RD values trends as PMI increases. These data were obtained by analyzing two different regions of the cardiac muscle: the apex and the septal region. More specifically, as PMI increases, it was possible to observe, on the one hand, the reduction of both the FA and the AD trends, while, on the other hand, the increase in values of both the ADC and the RD.

Apex FA dropped from 0.493036 ± 0.1648 at 3.5 hours (T0) to 0.404079 ± 0.1218 at 24 hours, showing a 18 % reduction. Septum dropped from 0.36935 ± 0.0984 at 3.5 hours (T0) to 0.329145 ± 0.0873 at 24 hours (T1), with a 11 % reduction.

Apex ADC increased from 0.463005 ± 0.1132 at 3.5 hours (T0) to 0.503881 ± 0.1031 at 24 hours, showing a percentage

Table 1. Change in FA, ADC, AD, RD values between 3.5 (T0) and 24h (T1) after death

	APEX (T0)	APEX (T1)	SEPTUM (T0)	SEPTUM (T1)
FA	0.493036 ± 0.1648	0.404079 ± 0.1218	0.36935 ± 0.0984	0.329145 ± 0.0873
	↓ 18%		↓ 11%	
ADC	0.463005 ± 0.1132	0.503881 ± 0.1031	0.474634 ± 0.0642	0.491062 ± 0.0840
	↑ 9%		↑ 4%	
AD	0.722619 ± 0.1231	0.721156 ± 0.1185	0.669492 ± 0.0985	0.650143 ± 0.0773
	↓ 1%		↓ 3%	
RD	0.333198 ± 0.1241	0.395244 ± 0.1090	0.38688 ± 0.0676	0.421847 ± 0.0856
	↑ 19%		↑ 9%	

change of 9 %. Septum increased from 0.474634 ± 0.0642 at 3.5 hours (T0) to 0.491062 ± 0.0840 at 24 hours (T1), showing a percentage change of approximately 4 %. Apex AD dropped from 0.722619 ± 0.1231 at 3.5 hours (T0) to 0.721156 ± 0.1185 at 24 hours (T1) (1 %). Septum dropped from 0.669492 ± 0.0985 at 3.5 hours (T0) to 0.650143 ± 0.0773 at 24 hours (T1). The percentage change was of approximately 1 % for the apex, 3 % for the septum.

Finally, Apex RD increased from 0.333198 ± 0.1241 at 3.5 hours (T0) to 0.395244 ± 0.1090 at 24 hours. Septum increased from 0.38688 ± 0.0676 at 3.5 hours (T0) to 0.421847 ± 0.0856 at 24 hours (T1). The percentage change observed was 19 % for the apex and 9% for the septum.

Tractography

The tractography study enabled the correlation of the aforementioned variations with a probable loss of both muscular fiber firmness and alterations in their structural architecture. In Figure 2, progressive fiber disorganization can be observed at T0 and T1 across the three cardiac projections: the short-axis view (A), and the long-axis views (B and C), obtained along the four-chamber (B) and three-chamber (C) planes, respectively. At T0, the organization of the myocardial fibers is coherent with the three-dimensional planes, and the myocardium looks compact and with a regular course. At T1, the degeneration of the myocardial

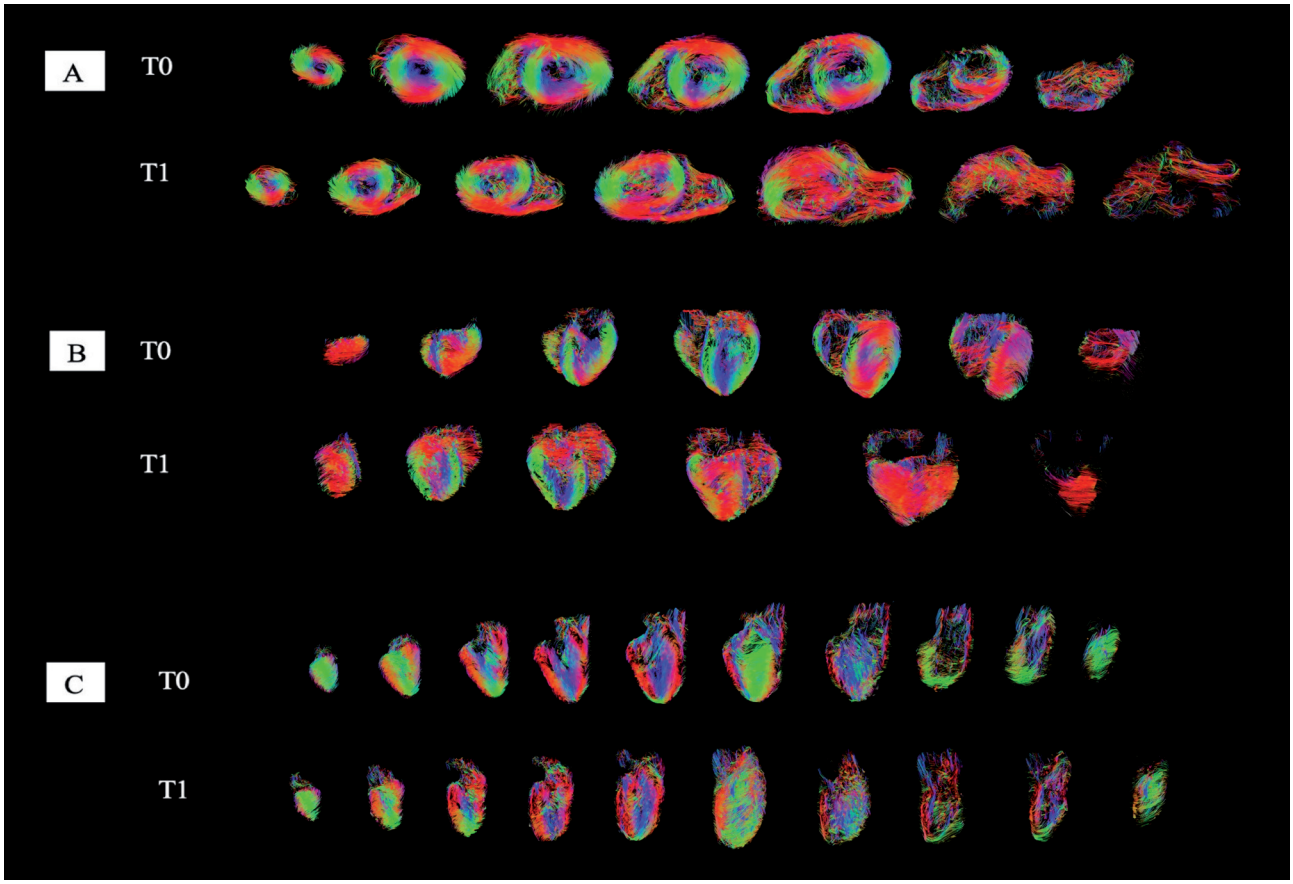

Figure 2. Tractography obtained from diffusion tensor sequence at 3.5 h (T0) and 24 h (T1) after death. All datasets were displayed along the cardiac short-axis (A), and long axis views, the latter respectively in the four-chamber (B) and three-chamber plane (C).

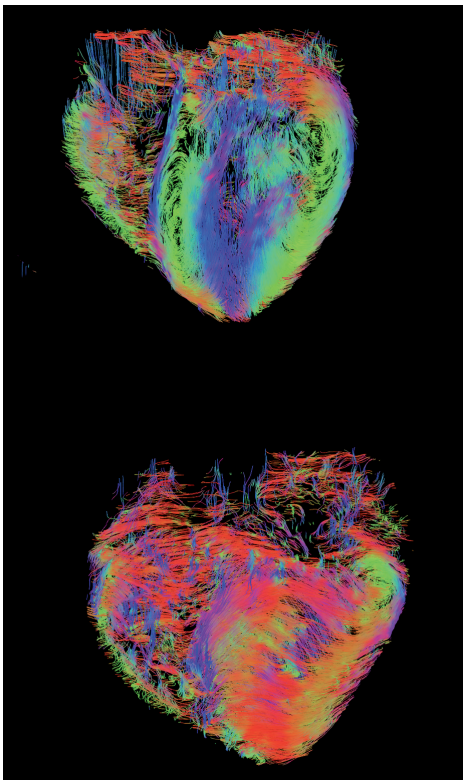

Figure 3. Cardiac horizontal long-axis view at times T0 (upper row) and T1 (lower row).

cells and their loss of organization is evident, due to their postmortem degradation (Figure 3).

Temperature

During the observational period, the heart temperature drops sharply in the first 10 h. Immediately after extraction, the heart temperature was 37.2°C. At the time of the first scan, 3.5 hours after death (T0), the recorded temperature was 33.8°C. The temperature measured 10 hours after death was 23.7°C. At 24 hours after death, prior to the second scan (T1), the temperature was 17.2°C, gradually approaching equilibrium with the external temperature (17.0°C).

The temperature was faster during the first 10 hours than in the subsequent 14 hours. These findings are consistent with the known pattern of postmortem cooling in human tissues (24,25).

Risk of bias

This study presents sources of bias that must be taken into account when interpreting the results because it was intended as a proof-of-concept (preliminary evaluation of technical applicability) rather than a validation study. First, only one pig heart was examined ex vivo and at two different times (3.5 and 24 hours postmortem) for this analysis. The observed changes in tractography and qMRMI/DTI parameters are therefore descriptive, and neither statistical inference nor temporal modeling are possible. Second, unlike the real-world forensic setting where the organ is in situ and postmortem kinetics are influenced by a variety of factors (cooling gradient, surrounding tissues, microbial dynamics), the experimental setup is purposefully simplified and controlled (constant temperature and humidity). All things considered, the findings should be seen as initial proof of viability, helpful in directing more extensive and practical research in the future.

Discussion

The ability to estimate the PMI as accurately as possible is considered one of the primary objectives of forensic pathology (2). In past years, various approaches have been evaluated for correctly estimating and calculating the PMI, without any success (3).

Despite that, in recent years the advancement of molecular biology techniques allowed to analyze new parameters, such as the degradation timing of proteins, DNA and RNA (26-28). These studies led to a further approach in the evaluation of PMI, through the use of qMRMI.

Based on the studies reported in international literature, it is possible to appreciate the efforts of the scientific community to implement virtual molecular imaging techniques in the forensic field, with the aim of achieving a more precise estimation of the PMI (26).

To date, the ePMI estimation only made use of physical postmortem alterations, such as those of body temperature, cadaveric rigidity, and the consequent relaxation of the muscles and/ or of the hypostasis observation: despite that, the application limits of these techniques have been widely described (2,4,29). From this perspective, the molecular imaging, making use of the so-called "quantitative magnetic resonance" focused on the study of tissue transformation related to the aforementioned degradation. Quantitative MRI evaluate both the electrolytic variations and the biochemical diffusion in tissues, leading to the acquisition of "molecular-tissue images". "This technique has already been applied in other fields of medicine, such as the study of cerebral ischemic consequences and cardiac amyloidosis, where the ADC, Fractional Anisotropy, and MT ratio are combined to develop a mathematical algorithm that allows images to be directly correlated with the quantitative effects of protein degradation (30-32). Molecular imaging has also been applied on animal models, for the evaluation of postmortem protein degradation, with considerable implications in PMI estimation (12).

Starting from these assumptions and considering what is already known in literature, authors have applied this technique for the evaluation of the myocardium protein degradation based on animal model, making use of a pig heart.

Anisotropic diffusion typically involves complex biological tissues that have different diffusion coefficients, in relation to their different space directions. As it happens in the striated skeletal muscle, where due to the parallel orientation of the fibers a high grade of anisotropy is found, similar findings can also be obtained in cardiac muscle (30).

Usually, the evaluation of anisotropic diffusion grade is obtained with the help of DTI, which is subsequently expressed via FA. Therefore, FA indicates the amount of asymmetric diffusion present in a voxel. The values within which it is possible to express the variation in FA are those

between 0 and 1: in particular, the closer this value is to 0, the greater is the grade of isotropic diffusion of the molecules. On the other hand, the closer this value tends to 1, the greater the grade of anisotropic diffusion will be (32). Among the factors that can influence the iso/anisotropopic diffusion of molecules, it has to be considered the integrity of cell walls: in fact, it is known that their alteration, such as flaking due to postmortem degradation, determines a reduction in FA (33,34).

Furthermore, since the DTI is strongly influenced by the density and orientation of the cellular structures that limit the water diffusion, it is possible to evaluate the microstructural characteristics of tissue of interest by means of fractional anisotropy: in our study it was possible to hypothesize a reduction in cardiomyocytes FA linked to the gradual destruction of PMI-related cell walls.

DTI images, obtained as aforementioned, can be reconstructed with a proper software, leading to the formation of tractographic images, which appear as colored fiber bundles.

Ultimately, the flaking of the cell wall is related to a decrease in FA, detectable as an alteration in tractography images. This structural alteration of the cardiomyocytes, due to their gradual disruption, is related to PMI, and it is appreciable in tractography, especially through the variation of fractional anisotropy. In our study, it was highlighted a reduction up to 18% at apex, and up to 11% at septal region.

These observations led us to hypothesized that there could be an association between quantitative MRI and a possible PMI estimation. For this reason, further studies will have to be conducted, using a larger sample and analyzing a greater number of intervals.

Limitations

This work aims to introduce an innovative approach (qMRMI/DTI and tractography) to the forensic settings for the study of early postmortem myocardial changes. However, several limitations limit its significance and prevent any immediate translation into routine practice. The study is based on a single porcine heart, acquired ex vivo and under controlled environmental conditions. This reduces variability and facilitates proof of principle, but does not reproduce the complexity of a real postmortem (organ in situ, nonlinear cooling, environmental variability, and decomposition processes). Temporal sampling is limited to only two acquisitions within the first 24 hours, making it impossible to describe the complete trajectory of changes and identify intermediate stages. Furthermore, the absence of cardiac pathologies in the experimental model limits generalizability to forensic settings, where fibrosis, ischemia, or cardiomyopathies can alter baseline parameters and confound interpretation. Finally, reproducibility, inter-operator variability, and operational feasibility (time, costs, scanner availability, and expertise) were not assessed. Future studies should include larger sample sizes, denser postmortem intervals, more realistic conditions (intact hearts with blood and/or whole cadavers), and pathology cohorts, ideally with histological and biochemical correlations.

Conclusions

This study, intended as a proof-of-concept, shows that the use of qMRMI/DTI and tractography is technically applicable to a controlled postmortem myocardial model and allows for the detection of measurable changes in microstructural parameters at least between 3.5 and 24 hours after death. These observations should be interpreted as preliminary and descriptive, useful primarily for introducing an innovative approach to the forensic literature and generating hypotheses on the temporal behavior of diffusion biomarkers in the myocardium. Given the single sample, the presence of only two time points, the ex vivo examination under constant environmental conditions, the results cannot be considered a validation or a method ready for routine use in ePMI estimation. Future studies should include larger samples, denser temporal sampling, and conditions closer to the real-world forensic scenario (intact organs with blood and/or whole cadavers), as well as assessing the impact of cardiac pathologies and operational feasibility. In this framework, qMRMI/DTI and tractography could represent a complementary support in selected cases in the future, once a systematic validation has been completed.

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