Observational Study

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Feasibility of dl-DECT for Postmortem Interval Estimation Through Changes in Yellow Bone Marrow: Preliminary Results

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Abstract

Being able to correctly estimate the post-mortem interval is one of the most challenging tasks in forensic pathology, as the most used methods are influenced by various factors that can reduce their reliability. Through this study, the authors propose an innovative method aimed to further narrowing the time interval within which to place the time of death. It is proposed an analysis of the yellow bone marrow using the dual-layer dual-energy computed tomog-raphy (dl-DECT) as a tool to potentially identify its alteration composition occurring post-mortem. In adults, in physiological conditions, DECT does not usually show fluid components within bone marrow. Therefore, the identi-fication of fluid-like components in the bone marrow of cadaveric femurs we observed could be attributable to post-mortem phenomena. The obtained results are still preliminary, therefore further studies are required. In the near future, further analyses will be performed by this research group, trying to identify a correlation between the variations of water in the yellow bone marrow and the time of death. ***Clin Ter 2026; 177 (4):705-710 doi: 10.7417/CT.2026.2061***

Keywords: *PMI estimation; postmortem interval estimation; forensic pathology; yellow bone marrow; dl-DECT; forensic radiology*

Introduction

Post-mortem interval represents the time interval between an individual death and its corpse first observation (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 (2,3). Given the ever-increasing need to reduce as much as possible the time interval within which to establish 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, as happened for the analysis of mRNA, miRNA and protein degradation, also evaluated through other radiological devices, such as PMMRI in animal models (4-7). Among these, the evaluation of the yellow bone marrow degradation pattern using dual energy computed tomography (DECT) could represent a new important tool.

Computed tomography (CT) is a diagnostic imaging method that makes use of X-rays for reproducing, in an extremely similar way, both the anatomy and the morphological alterations of body' organs and tissues (8).

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CT measures the attenuation of X-rays as they pass through human tissues, which is recorded by a system of detectors that rotate around the patient. A numerical value, defined CT number, is assigned to each pixel of the image, representing the average of all the attenuation values contained within the corresponding unit of the studied volume (voxel). The measured attenuation of a tissue is then scaled to reference substances (water and air) and displayed on a scale of arbitrary units named Hounsfield units (HU) (9).

A limit of traditional CT scans is the fact that different tissues may have similar densities and present overlapping HU values at a given peak tube voltage, making them impossible to distinguish.

A relatively recent advance of CT technology is represented by dual-energy CT (DECT), also referred as spectral CT, which provides information about how substances behave at different energy levels (high and low energy levels).

Several DECT technologies currently exist, which operate either at the source level (like dual-source CT, where two tubes running at different voltages generate two different X-ray spectra) or at the detector level (like dual-layer CT, with a single X-ray tube but two layers of detectors with maximal sensitivities to different photon energies).

By analyzing energy-dependent changes of the X-ray attenuation of materials, DECT makes it possible to obtain additional information about tissue density and atomic number that can be used to distinguish tissues and materials which in a conventional CT image present similar attenuation and HU values and are therefore difficult to differentiate (10,11).

Several DECT algorithms exist aiming to separate materials and extract quantitative information from the acquired data.

Among these, Z-effective (Zeff) maps are color-coded images based on the effective atomic number (Z) of materials. These maps provide a higher level of discrimination of tissues compared to conventional CT images, since they provide the material make-up of each pixel (10).

Other algorithms frequently used - alone or in combination with others - in musculoskeletal imaging are techniques designed to selectively remove or reduce the signal from calcium, like virtual non-calcium (VNCa) and Calcium Suppression (CaSupp) techniques. By reducing the visualization of cancellous bone, these techniques permit a better assessment of bone marrow and its alterations (like the presence of bone marrow edema) in several osseous districts (12,13).

In this preliminary report, we performed a postmortem evaluation of femoral bone marrow in two subjects by means of Zeff maps (alone and in combination with CaSupp images) derived from dual-layer DECT (dl-DECT) as a potential tool to identify bone marrow alteration composition occurring after death.

2. Materials and Methods

The right femurs of a 29 and 40-year-old men were analysed with dl-DECT before autopsy. The yellow bone marrow radiological examination was performed respectively 3 days and 8 hours and 4 days and 3 hours after the first observation of the corpse (discovery at the scene). Before undergoing the CT examination, the bodies were kept in a refrigerated mortuary chamber for 19 and 26 hours, respectively, at a temperature of 4 °C.

Both patients underwent the same CT protocol, which consisted of a total-body unenhanced scan performed on a DL-DECT platform (IQon Spectral CT, Philips Healthcare, Best, The Netherlands).

Acquisition parameters were set as follows: tube voltage 120 kVp with automatic modulation of mAs; nominal layer thickness 0.67 mm; scan interval 0.34 mm; detector collimation 64 × 0.625 mm; tube rotation time 0.27 s; matrix 512 × 512, field of view: variable according to patients' characteristics.

Axial, coronal and sagittal reconstruction were then obtained (slice thickness: 2 mm; increment: 1 mm).

Spectral-CT data of both CT scans were exported as spectral base image (SBI) files, which were then sent to a dedicated workstation (IntelliSpace Portal v.8.0, Philips Healthcare, Best, Netherlands) to obtain Zeff color-coded maps.

Image evaluation was then performed on the right femurs of both patients.

First, Zeff maps and superimposed Zeff and CaSupp (Zeff-CaSupp) color-coded maps were assessed for a visual general analysis of femoral bone marrow and its composition.

Subsequently, a quantitative evaluation was performed. Image segmentation was conducted and specific volumes of interest (VOIs) encompassing most of the femoral shafts were selected (from a horizontal line drawn 5 cm distal to the lesser trochanter to a horizontal line drawn 10 cm above the femoral condyles).

For the segmented VOIs, Zeff values were derived and relative histograms were plotted.

For Zeff maps, each voxel is set to a value in variable ranges reflecting the effective atomic number of that voxel. According to the dl-DECT manufacturer used in this study, the following Zeff reference values were used: fat = 5.9, water = 7.4, cortical bone = 13. For trabecular bone, the reference value was set as 8.3. Therefore, areas with Zeff below 7.4 were considered compatible with adipose tissue (consistent with yellow bone marrow), components with Zeff between 7.4 and 8.3 with fluid, while those above 8.3 with trabecular and cortical bone.

3. Results

3.1. Subject 1

The case concerns a 29-year-old male with a history of substance use disorder and previous admission to a rehabilitation facility.

The subject was found unresponsive at home. Emergency services attempted resuscitation without success, and death was pronounced at the scene. Circumstantial findings included drug paraphernalia consistent with intravenous administration of narcotic substances.

Autopsy revealed multiple injection marks with recent hemorrhagic infiltration, pulmonary congestion and edema, and evidence of acute myocardial injury in a heart with preexisting left ventricular hypertrophy and advanced coronary atherosclerosis. Histological analysis confirmed acute pulmonary edema and multifocal myocardial necrosis.

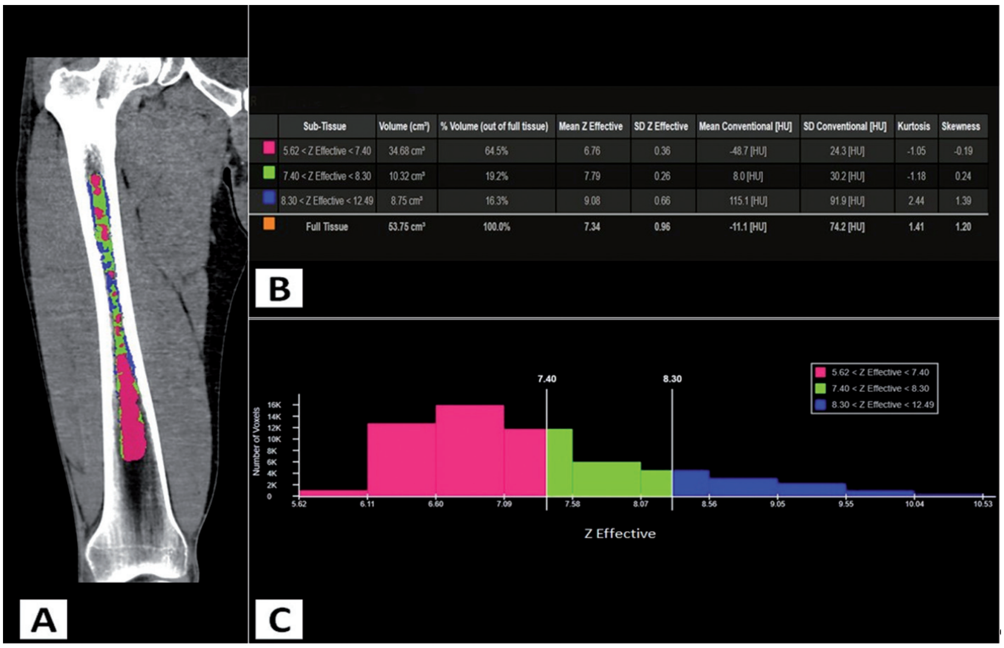
Toxicological investigations detected metabolites of heroin (morphine and 6-MAM) as well as cocaine and benzoylecgonine in the blood, confirming a recent combined intake of these substances.

Death was attributed to cardio-respiratory arrest due to acute intoxication from combined heroin and cocaine use, with pre-existing structural cardiac pathology acting as a contributing factor.

The estimated time of death was placed within a few hours after the subject was last seen alive, and 3 days and 8 hours before CT was performed.

CT visual (Fig. 1) and quantitative (Fig. 2) evaluation was performed on the patient's right femur.

Figure 1. (A) Z-effective (Zeff) image. (B) Superimposed Zeff and CaSupp (Zeff-CaSupp) color-coded maps. Two ROIs were delineated in distinct areas of the bone marrow that exhibited different atomic number values. The ROI in the blue area had a Zeff value of 6, while the ROI in the green area had a Zeff value of 7.4, corresponding to adipose and fluid-like tissue, respectively.



Sub-Tissue	Volume (cm³)	% Volume (out of full tissue)	Mean Z Effective	SD Z Effective	Mean Conventional [HU]	SD Conventional [HU]	Kurtosis	Skewness
5.62 < Z Effective < 7.40	34.68 cm³	64.5%	6.76	0.36	-48.7 [HU]	24.3 [HU]	-1.05	-0.19
7.40 < Z Effective < 8.30	10.32 cm³	19.2%	7.79	0.26	8.0 [HU]	30.2 [HU]	-1.18	0.24
8.30 < Z Effective < 12.49	8.75 cm³	16.3%	9.08	0.66	115.1 [HU]	91.9 [HU]	2.44	1.39
Full Tissue	53.75 cm³	100.0%	7.34	0.96	-11.1 [HU]	74.2 [HU]	1.41	1.20

Figure 2. (A) Resulting image from the 3D segmentation analysis. (B) Table showing the volume (expressed as both absolute values and percentages), the mean Z-effective value of each tissue, and the mean conventional at-tenuation in HU. (C) Histogram showing the results of spectral sub-segmentation.

3.2. Subject 2

The case concerns a 40-year-old male with no relevant past medical history and no evidence of chronic or acute disease.

While performing construction work on a roof, the subject was accidentally struck by a moving beam, which caused a loss of balance and a fall from an estimated height of 6–7 meters. During the fall, he landed backwards on the free end of a metallic scaffolding tube, resulting in a passive impalement through the right thoraco-lumbar region.

Autopsy revealed a penetrating trauma with an intracorporeal track exceeding 20 cm, lacerating the retroperitoneum, right kidney region, liver, diaphragm, and entering the right pleural cavity. More than 2 liters of blood were found in the pleural space, along with additional hemoperitoneum.

Death occurred rapidly due to cardio-respiratory arrest from hemorrhagic shock. The estimated time of death was consistent with the timing of the traumatic event and the immediate emergency assessment, supporting a recent death following the injury.

There was no evidence of pre-existing disease that could have contributed to the fall or the fatal outcome. The manner of death was classified as accidental, occupational injury. The subject died approximately 4 days and 3 hours before the radiological examinations.

The same procedure used for the first individual was applied (Figure 3 and Figure 4). The total volume of segmented bone marrow was 28 cm^3. This is noteworthy given the individual's physical conformation, as the femoral medullary canal was reduced in size. The fluid component accounted for 47.5% (13.30 cm^3), the adipose tissue component for 35.4% (9.93 cm^3), and the trabecular bone component for 17.1% (4.79 cm^3).

Proportionally, although the segmented bone marrow volume is reduced, the aqueous component is increased in the second cadaver. This finding is relevant to the postmortem interval, as the close temporal proximity to death suggests that postmortem changes may already be advanced.

4. Discussion

In human body it is possible to distinguish between two types of bone marrow: red bone marrow and yellow bone marrow (14). The red bone marrow is approximately made up of 40% water, 40% fat and 20% protein (14). Yellow bone marrow is instead made up of 80% fat, 15% water and 5% protein.

During life, the red bone marrow undergoes continuous transformation phenomena which determine its progressive transformation into yellow bone marrow (14). The first transformative phenomena begin already during the last months of gestation (14). In particular, the conversion proceeds from appendicular to axial skeleton, and from the diaphysis to epiphysis (14,15). These phenomena make it difficult to use red bone marrow for the purposes of this study, as it would be necessary to standardize its composition by age and sex.

On the contrary, yellow bone marrow is already well represented at a young age within some districts, such as that of the lower limb, and in particular in tibia and femur. Therefore, in adults femoral bone marrow is normally composed almost entirely of adipose tissue; however, post-mortem changes can lead to an increase in its water component.

The findings of fluid within yellow bone marrow in an adult femoral bone of a cadaver could represent a sign of the degradation of bone marrow, thus potentially representing a tool to establish PMI.

The gold standard technique for studying the bone marrow is represented by MR imaging (14).However, MR

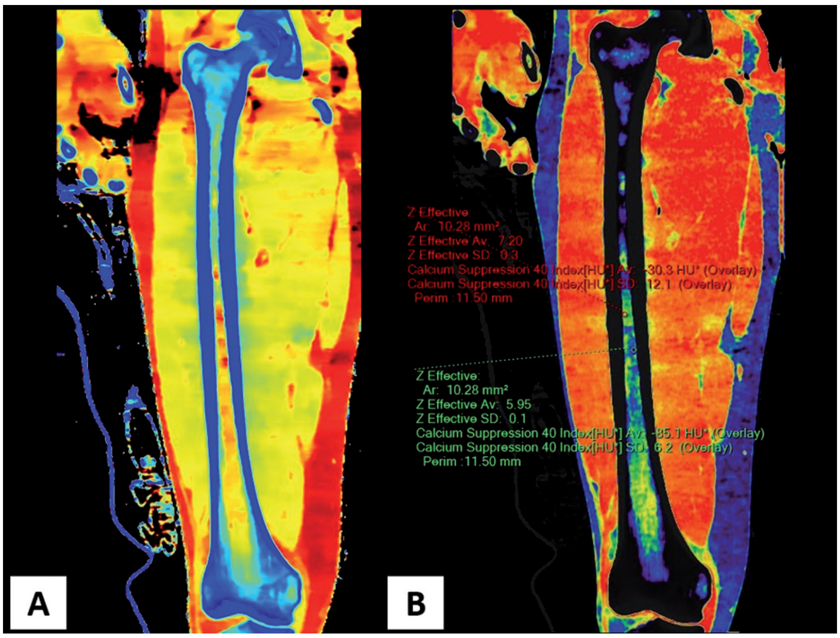


Figure 3. (A) Z-effective image (Zeff). (B) Superimposed Zeff and CaSupp (Zeff-CaSupp) color-coded maps showing two different ROIs placed in the watery component (green) and adipose component (red) of the bone marrow in the right femur.

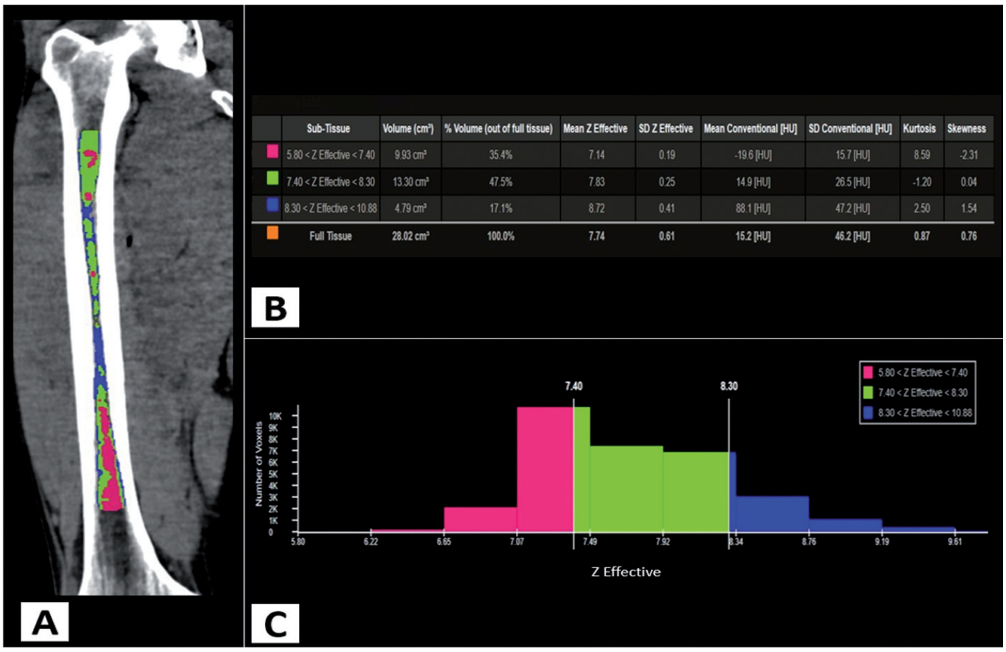


Figure 4. (A) Result of 3D segmentation. (B) Table showing the results of spectral sub-segmentation. (C) Histogram of spectral sub-segmentation results.

imaging is a time-consuming and expensive technique to use in the evaluation of bone marrow to establish the time of death in cadavers (15). Instead, DECT could be a promising tool as it can identify an increase in watery content within bone marrow and may also be useful in determining the cause of death (15).

In this preliminary report, the main added value of dl-DECT is the possibility of moving from a qualitative impression of marrow "watery content" to a measurable, volumetric endpoint derived from routinely acquired post-mortem CT data.

Zeff images derived from dl-DECT showed different components within bone marrow in the evaluated cadavers in a color-coded scale. Specifically, Zeff-CaSupp maps, due to the removal of the calcium component of the attenuation, were ideal for the visual distinction between fat (representative of normal yellow marrow) and fluid (possibly consistent with degenerated edematous marrow).

Quantitative evaluation of femoral shaft showed that the Fluid-like component made up 19.2% (10.32 cm³) of the segmented marrow volume in Subject 1 and 47.5% (13.30 cm³) in Subject 2, with a similar trabecular-bone fraction (~16–17%) that might be partially due to partial-volume effects at the endosteal boundary. Although no temporal inference can be drawn from two cases, the marked difference in aqueous fraction despite a similar postmortem time window suggests that marrow spectral changes may be strongly modulated by case-specific variables, such as agonal events, cause of death, post-mortem handling and environmental conditions, and therefore should be investigated within larger cohorts with structured data. These data support feasibility, but also underscore the need for standardized segmentation rules, reproducibility testing, and robustness analyses of threshold selection.

4.1. Risk of bias and future perspectives

Independent of PMI, several factors may affect the composition of bone marrow and the detectability of a fluid-like component. First, the balance between fatty and cellular/aqueous marrow may be altered by antemortem marrow variability, such as age, sex, body habitus, smoking status, chronic hypoxia, systemic inflammation, anemia, or occult hematologic disorders. Therefore, these variables should be methodically documented and, if feasible, excluded or modeled as covariates. Second, intramedullary fluid redistribution and decomposition-related changes may be accelerated or slowed by peri- and post-mortem conditions, such as environmental temperature, refrigeration, humidity, clothing/insulation, body position, and time from discovery to transport and imaging.

Future research should therefore include structured documentation of storage conditions and take temperature-adjusted models into consideration. Third, stratified analyses by major cause-of-death categories will be required because the cause of death and agonal events, such as massive bleeding, shock, sepsis, intoxication, prolonged hypoxia, may change tissue hydration or vascular permeability as well as post-mortem marrow behavior.

It is also important to take technical confounders into account.

In the literature there is no standardization of Zeff values in relation to tissue density. We used the manufacturer-reported reference values for fat, water and cortical bone (16). The trabecular region is a mixture of bone and marrow impossible to separate within the single voxel; for this reason, there is a wide range of the reported Zeff values in the literature for trabecular bone, also reflecting the specific anatomic region in study depending on the relative proportions of marrow and mineralized bone (with higher Zeff values where the cancellous structure is denser and lower Zeff values in marrow-dominant regions) (17,18).

We therefore used a Zeff value relatively low, where the cancellous structure is less dense (like in the femoral shaft).

This high variability and the fact that many tissues have similar Zeff values represents the main limitation for tissue differentiation with this algorithm. Furthermore, vendor-specific settings, calibration and reconstruction parameters, partial-volume effects, motion or post-mortem gas artifacts can all affect Zeff measurements. To lessen reliance on fixed cut-offs and better handle “borderline” voxels impacted by noise or partial volume, future research will include phantom-based calibration and sensitivity analyses investigating threshold perturbations as well as alternative classification techniques (e.g., probabilistic mixture modeling of the Z-effective histogram). Additionally, standardizing the segmented segment length and anatomical landmarks is anticipated to decrease variance and enhance comparability across individuals because the precise boundaries of the segmented medullary volume may differ depending on anatomy and operator decisions.

Conclusions

The feasibility of employing dl-DECT post-processing to visualize and measure compositional changes within adult femoral yellow bone marrow in the post-mortem setting is supported by this preliminary study. We were able to document a measurable fluid-like component within the medullary compartment in two cadavers and estimate the relative fractions of fat-like, fluid/soft-tissue-like, and bone components by combining Zeff mapping, CaSupp fusion, and 3D volumetric segmentation.

However, because of the extremely small sample size, the lack of a comparative reference standard, and the possible impact of ante-mortem variability, environmental conditions, and technical factors affecting spectral measurements and segmentation, the current results must be interpreted cautiously. A larger, methodically characterized cohort with standardized segmentation rules, organized documentation of post-mortem handling and temperature-related variables, and a formal evaluation of inter-/intra-observer reproducibility will be the next stage. Prior to assessing the potential of this method as an additional tool in forensic casework and testing strong statistical correlations between marrow aqueous fraction and PMI, these developments are required.

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