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Using histology to evaluate micro-CT findings of trauma in three post-mortem samples — First steps towards method validation

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ABSTRACT

Forensic imaging technology has rapidly advanced over the past several decades and is gaining increasing significance in medico-legal death investigations. Medical-grade computed tomography (CT) is now routinely used in post-mortem examinations at numerous institutions across the globe. However, the resolution of medical-grade CT is limited and unsuitable when used to depict some smaller anatomical structures or micro-trauma. High-resolution micro-CT offers up to 100× the resolution to overcome this problem but is a very recent addition to the field of forensic radiology. Few studies so far have attempted to validate the results which is an essential prerequisite for it to be used in the criminal justice process as demanded by regulatory bodies. This study directly compares micro-CT images with histology, the current gold standard. Three cases were examined: two larynges from suspected strangulations and one ribcage of a case of fatal child abuse. A strong correlation was observed between histology and micro-CT as the majority of skeletal injuries were identified correctly. This paper discusses the forensic implications of the results and how micro-CT is complementary to histology.

1. Introduction

The use of digital imaging technologies has gained increasing significance as an integral part of forensic post-mortem examinations over the past several decades, leading to the development of the sub-discipline of forensic radiology [1]. These technologies include Magnetic Resonance Imaging (MRI), computed tomography (CT), radiography, ultrasound, and surface scanning, although a review by Baglivo et al. [2] clearly demonstrates the popularity of CT over other methods. With the number of clinical autopsies declining often due to cultural and religious reservations [3], more and more researchers explore the (partial) replacement of full autopsies with less invasive methods [4,5]. Comparisons between these proposed methods and existing ones are therefore needed. This problem has been addressed by several studies which reach the broad consensus that CT is equal or superior to standard autopsy for skeletal injuries but inferior for organ and soft tissue trauma [6–8]. While the documentation of skeletal injuries certainly is improved by CT [2,9] hospital CT scanners have a limited resolution and therefore might fail to detect more subtle injuries which has led to increasing recognition of micro-CT (μCT) as a useful imaging modality [10–12] although its availability remains limited.

Micro-CT is frequently used in industry for non-destructive inspection of internal structures of small manufactured parts or the internal characteristics of different materials [13] where the resolution of standard CT is insufficient to visualise such small detail. The higher resolution required has been achieved in part by higher power as there is less concern for radiation than in hospital setups and longer exposure times as there are no artefacts created by patient movement [14]. Concerns of dose and motion artefacts are less applicable to the study of cadaveric tissues which has increased the number of studies using post-mortem CT for medico-legal death investigations [1,2]. Micro-CT in particular has enabled investigators to focus on individual injuries in more detail which has proved particularly helpful for the assessment of paediatric rib fractures [12]. The unprecedented level of detail which this novel technology is able to produce brings exciting new possibilities but also presents challenges as the interpretation of the images becomes more difficult. This paper therefore aims to use histology, the current gold standard used in forensic wound analysis for fracture identification and fracture age determination [15], to assess the findings observed on the micro-CT scans. Not only is this

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essential for making sense of the unfamiliar data but it is also a crucial part of the validation process required for the method to be used in a forensic context. Validating forensic science methods has become an increasing concern across the forensic community since the Forensic Science Regulator has introduced ISO accreditation as a requirement for all forensic laboratories, promising higher quality standards in the field [16]. Micro-CT has been demonstrated as a suitable imaging method in industrial settings [17,18] but few studies have compared its reliability and accuracy to existing medico-legal techniques [19,20]. If micro-CT is ever to be used independently it must be proven to produce reliable results comparable to histology with known limitations and error rates. This study aims to present the first steps towards demonstrating the method's suitability to identify trauma by comparing findings from two imaging modalities: micro-CT and histology.

2. Materials and method

Three different specimens were examined for this study: two adult laryngo-hyoid complexes from cases of suspected strangulation, and one five-months-old's ribcage from a case of child abuse. All specimens were sampled *en gros* during forensic post-mortem which was conducted within a day of death. They were fixed in formalin for a few days to prevent tissue degradation before being submitted for micro-CT scanning.

2.1. Micro-computed tomography (micro-CT)

Micro-CT scanning involves taking radiographs of an object at angular increments through a full 360° rotation. The object is placed on a rotating table in the X-ray beam path emitted by the source. Opposite the source is the detector which detects the X-ray signal after it has travelled through the object resulting in individual grey-scale radiographic projections that are then reconstructed to build a 3D volume of the scanned object [21]. The typical setup of a lab-based micro-CT system is shown in Fig. 1.

The complete samples were removed from the formalin solution into which they had been placed at the mortuary in preparation for the micro-CT (μCT) examination and placed into foam-padded sealed plastic containers. All scans were conducted by researcher 1 using a Nikon XT 225/320 LC (Nikon Metrology, Tring, UK) using scan parameters detailed in Table 1.

The CT scans were reconstructed using the system's proprietary software CTPro 3D and viewed in VG Studio MAX 2.2 (Volume Graphics, Heidelberg, Germany), an inspection software for CT data.

2.2. Histology

Directly following the micro-CT examination the samples were submitted for histological analysis which was performed by researcher 2 who had access to the micro-CT report. Initial examination included radiographs of the sample and a macroscopic inspection before decalcifying the samples in 1:1 formic acid/formalin solution for up to several weeks depending on the sample size. The samples were then dissected and examined for tissue block selection for processing to paraffin in order to be sectioned on a Leica RM 2255 microtome (Leica Biosystems UK, Milton Keynes, UK). Sections were stained using haematoxylin and eosin (H&E) stains (Leica Biosystems UK, Milton Keynes, UK) and Martius Scarlet Blue (MSB, Atom Scientific, Manchester, UK) where indicated. H&E was chosen as it is the standard histological stain used in the broad field of histopathology, and the trichrome MSB because it detects the fibrin mesh that forms in the haematoma which develops around fractures. The larynges were sliced in the transverse plane at intervals of 5–10 mm. Each slice was photographed and processed in megablocks for histology. From the ribcage individual ribs were dissected free and blocked out in their entirety. Microscopic analysis was performed using an Olympus BX53 light microscope (Olympus, Southend-on-Sea, UK).

Both μCT and histology sections were inspected visually by researchers 1 and 2 respectively. The criteria used to examine the micro-CT scans were: fractures of the ossified tissue visible in both the volume rendering and the 2D sections, the appearance of the edges of such fractures (smooth v sharp), fissures through the cartilage of the larynx, evidence of new bone formation, and haematoma. The histology slides were examined for the presence/absence of the fracture, the type of fracture (e.g. partial, complete, displaced) and the presence/absence of a local tissue response to the fracture and associated haematoma. The tissue reaction progresses through resorption of the haematoma and dead bone followed by the step-wise and orderly repair through granulation tissue formation, fibrosis, callus formation and resolution to the healed state. The stage reached at which this tissue response is interrupted by death is the basis for estimating the age of the fracture at the time of death. A second reading of the micro-CT results was conducted by researcher 1 after consulting the histology report. As a study by Walton et al. [22] shows, the radiation produced during μCT scanning does not alter a sample's cell structure which justifies the sequence of examinations taken in this study.

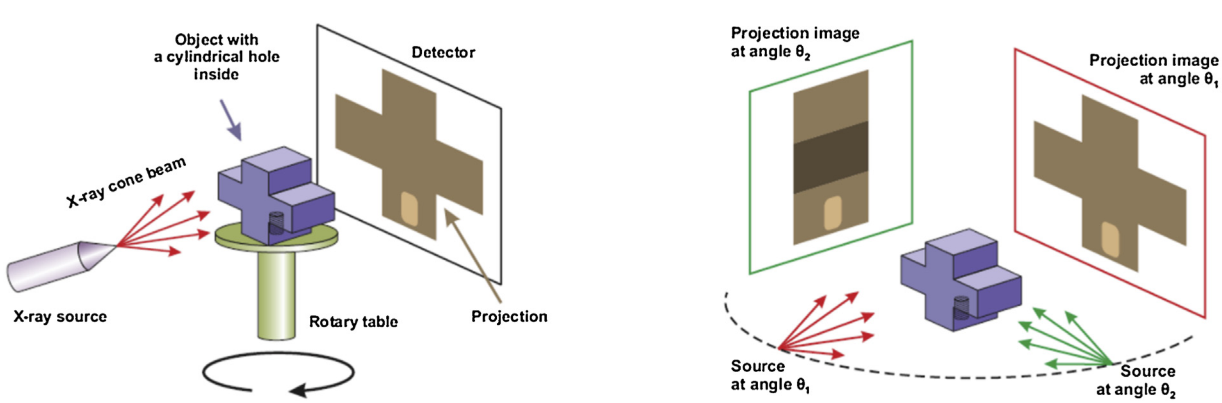

Fig. 1. Typical set-up of a lab-based micro-CT scanner.

Table 1
Scan parameters employed in this study; all scans were conducted on a Nikon XT 225/320 LC micro-CT scanner.

Sample	Voltage (kV)	Current (μA)	Exposure (ms)	Filtration	Gain (dB)	Number of projections	Voxel size (μm)
Larynx 1	80	718	708	0.35 mm copper	18	3141	57.5
Larynx 2	150	73	500	0.35 mm copper	24	3141	79.7
Ribcage	150	213	354	–	24	3141	94.9

3. Results

3.1. Case one: adult larynx 1 (male, 36 years)

3.1.1. Micro-CT

The analysis of the micro-CT images revealed one possible and two definite injury sites, shown in Fig. 2. Clear fractures were observed on the posterior right greater horn of the hyoid (area A) and through the ossified cartilage on the inferior border of the thyroid laminae to the left of the anterior midline (area B). The ossified cartilage surrounding the fracture on the left side displays smooth edges and a thickened region, which was cautiously interpreted as an irregular ossification pattern. An area of possible damage was observed at the base of the left superior horn of the thyroid cartilage (area C) which appeared as a fracture through the ossified cartilage but the overall irregular ossification pattern made a definite diagnosis difficult.

3.1.2. Histology

A fracture was detected involving the right greater horn of the hyoid bone associated with osteocyte necrosis either side of the fracture line, little haemorrhage, and intact periosteum. There was no inflammatory or mesenchymal tissue response. Fig. 3 directly compares the micro-CT and histological appearance of this fracture.

A displaced fracture was identified on the left superior horn of the thyroid cartilage containing fibrin and showing adjacent osteocyte necrosis, osteoclast activity with osteoblast reaction, and mesenchymal tissue response including an area of intramembranous reaction. The displacement of the superior horn fracture was attributed to the sectioning process (cf. micro-CT appearance).

Inferior to that was a further fracture through the thyroid cartilage on the left posterior part which did not disrupt the perichondrium and contained a limited amount of haematoma with no inflammatory or mesenchymal reaction.

Fig. 2. Antero-lateral view of the volume-rendered μCT scan of larynx 1 showing all the injuries identified initially: A = hyoid fracture, B = fracture on left inferior margin of thyroid lamina, C = possible fracture of left superior thyroid horn.

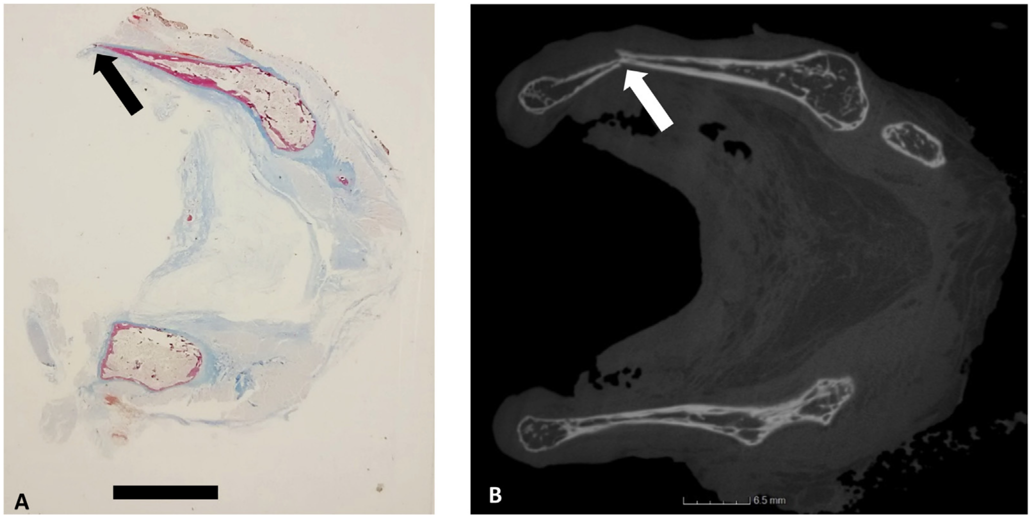

Fig. 3. Comparison between A: histology slide (MSB stain, scale bar = 10 mm) and B: micro-CT section (right) through the fracture on the right greater horn (arrow) of case 1.

One level below, on the inner surface of the posterior part on the right side of the thyroid cartilage, a small sub-perichondrial haematoma comprising phagocytic inflammatory cells and adjacent necrotic perichondrium was observed. On the outer subperiosteal bone opposite this haematoma, there was an incomplete fracture through the endochondral ossification showing osteocyte necrosis and medullary haemorrhage and minimal early periosteal reaction.

Soft tissue haemorrhage was observed, particularly in the left side associated with fat necrosis and minimal tissue reaction such as the presence of phagocytes and an early mesenchymal reaction.

3.2. Case two: adult larynx 2 (female, 39 years)

The results from all imaging modalities used in this case are compared in Fig. 4A–F.

3.2.1. Micro-CT

The scan images showed fractures at the bases of both superior thyroid horns with displacement of the horns anteriorly and medially (F). The trachea appeared partially severed approximately 30 mm from the top. A small circular cartilage defect was seen on the right thyroid lamina but this was interpreted as a natural feature, possibly a vessel opening.

3.2.2. Histology

Radiographs taken prior to the histological examination showed a discontinuity and displacement of the right superior thyroid horn (A). Macroscopic inspection detected haemorrhage into the right sternohyoid and omohyoid muscles (B–D) and a post-mortem autopsy cut on the trachea.

Microscopically, there was extensive fresh haematoma without tissue reaction in the sternohyoid and omohyoid muscles.

A fracture through the cartilage was observed on the left superior horn. The tip of the right superior thyroid horn was seen to be separated from the lower part although the fracture line was not observed in the plane of section. The displacement of the fragments and associated haemorrhages were taken as evidence of a fracture (E).

The lower sections through the trachea revealed extensive bilateral fresh haemorrhage into the respiratory mucosa and congested vessels.

3.3. Case three: juvenile ribcage (female, 5 months)

3.3.1. Micro-CT

Two subtle fractures were identified on the anterior ends of the 6th and 7th left ribs. Both fractures involved the costal cartilage and displayed a detached triangular fragment.

3.3.2. Histology

Initial radiographs taken did not show any fractures and macroscopic inspection did not show any definite fractures. Histology of individual ribs revealed a total of eleven fractures of mainly two types — anterior fractures through the costo-chondral junction on ribs L4-7 and posterior deep osteocartilaginous corner fractures on ribs R4-5, R8, R10, and L7-8. Fig. 5 shows the posterior fracture through the right fifth rib as an example. There was also a small cortical rib shaft fracture on the anterior right 4th rib.

All but one (L6A, complete, shown in Fig. 6) of the fractures were partial and non-displaced. The anterior fractures displayed primary spongiosa trabecular discontinuity, limited haemorrhage, fibrin deposition, osteocyte necrosis adjacent to the fracture line, an early limited tissue response and no significant disturbance of the growth architecture of the adjacent costal cartilaginous tissue. The posterior fractures were all on the deep corner of the osteocartilaginous interface of the rib head. They were predominantly associated with fibrin deposition, primary spongiosa trabecular discontinuity, and subtle and patchy osteocyte necrosis.

4. Direct method comparison and second reading

4.1. Method comparison

Out of the total of fourteen bone fractures, micro-CT detected five initially and a further eight after the second reading, summarised in Table 2. Micro-CT identified one additional feature which was not identified as trauma histologically. In case 3, the two fractures initially described on the micro-CT images were the two most prominent ones, labelled complete and partial in the histology report. The remaining ones were described as subtle or micro-fractures and were only identified on micro-CT following the histological assessment. None of the soft tissue injuries could be identified on the scans, even after the second

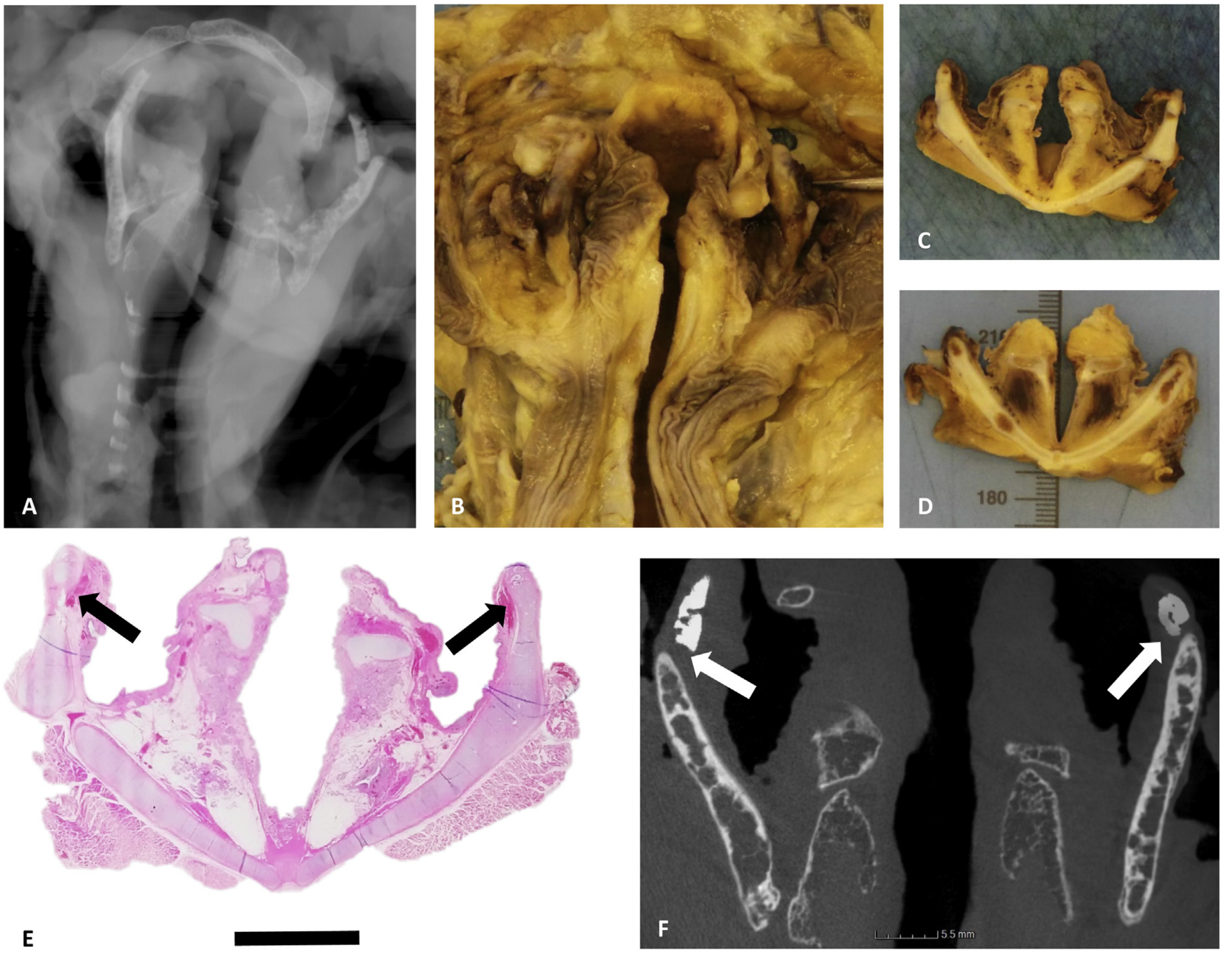

Fig. 4. Comparison between the different levels of examination. A: radiograph; B: macroscopic image, seen from posterior. The arrows indicate the areas of haemorrhage; C + D: transverse sections through the thyroid cartilage, the arrows indicate areas of haemorrhage; E: histology slide, H&E stain; scale bar = 10 mm. Areas of haemorrhage are indicated by the arrow heads; F: micro-CT section, seen from posterior. The arrows indicate the displaced fractured superior thyroid horns.

reading. This is due to the insufficient image contrast between different soft tissues. This became evident in cases 1 and 2 as the laryngeal cartilages and muscle tissue could not be visualised sufficiently. In cases of possible strangulation, cartilaginous trauma and haemorrhaging into the neck muscles are important indicators of compression to the neck [23]. This emphasises the complimentary nature of the two methods employed. Some researchers have addressed the problem of soft tissue contrast by introducing contrast agents [24,25] but this causes potential further problems relating to reversibility and effects on the subsequent histological examination and is difficult to implement in practice. Advancements in CT technology will help to overcome this issue and allow better differentiation of different soft tissues [22,26].

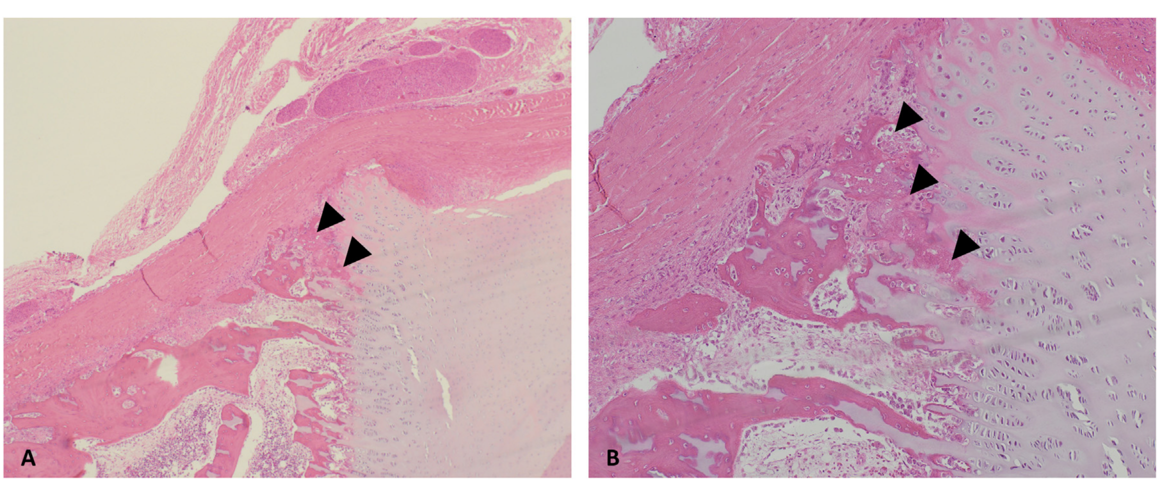

Fig. 5. Posterior corner fracture through the right fifth rib A: ×2 objective; B: ×10 objective; H&E stain. The arrow heads indicate the fracture.

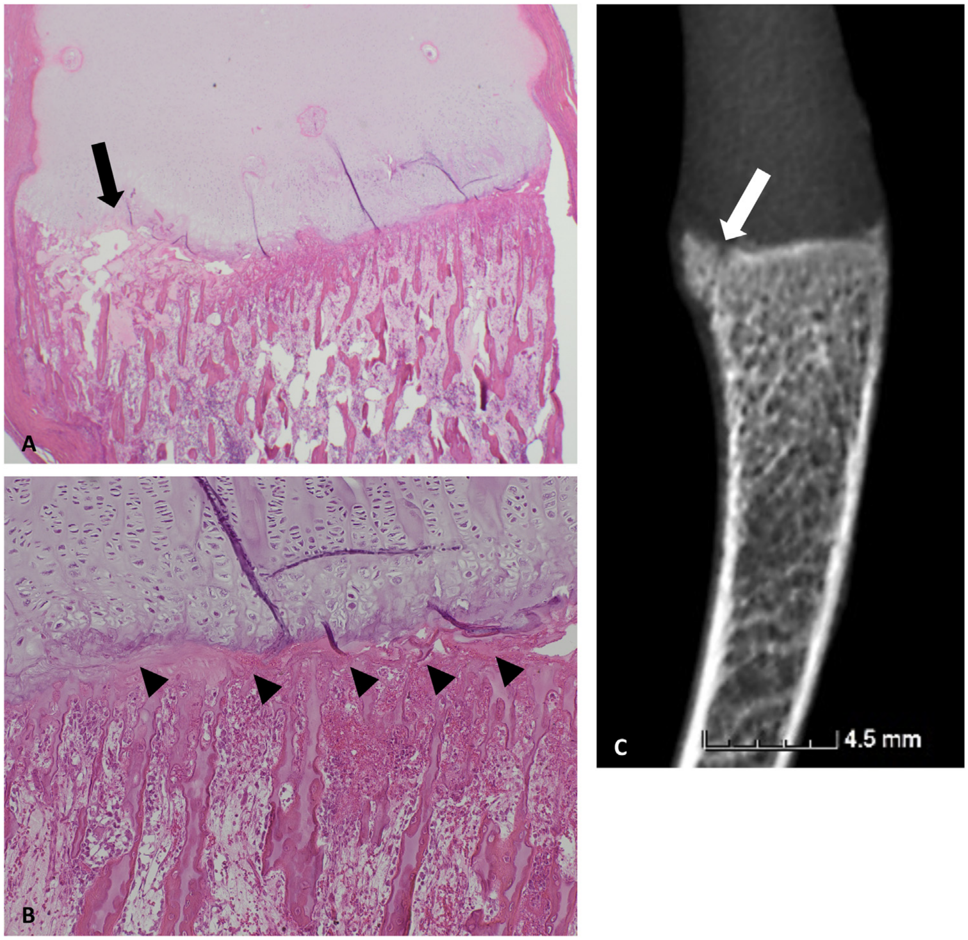

Fig. 6. Histology slide showing the anterior fracture of the left 6th rib. A: ×2 objective, B: ×10 objective; H&E stain. Through the ×10 objective the fracture can be clearly seen to run along the costo-chondral junction (arrow heads). C: micro-CT image of the injury (arrow).

Table 2
Overview of the features observed using both imaging modalities. The features in italics in the micro-CT column were only identified with certainty after viewing the histology report.

Injury location	Histology	Micro-CT
Case 1		
R greater horn	Fracture with osteocyte necrosis, little haemorrhage, intact periosteum, no inflammation	Fracture with sharp edges
Base L superior thyroid horn	Displaced fracture containing fibrin, adjacent osteocyte necrosis, osteoclast activity and osteoblast response, mesenchymal tissue response	Possible non-displaced fracture at base of left superior thyroid horn
L inferior border thyroid lamina	Not detected	Fracture with rounded edges, thickening around the fracture
L post thyroid cartilage	Fracture with intact perichondrium, limited haematoma, no inflammation	Not detected
R post thyroid cartilage	Incomplete fracture with osteocyte necrosis, medullary haemorrhage, early periosteal reaction, nearby sub-perichondrial haematoma	Not detected
Case 2		
Base L superior thyroid horn	Cartilage fracture	Displaced fracture with sharp edges
Base R superior thyroid horn	Separation with associated haemorrhage	Displaced fracture with sharp edges
Trachea	Autopsy cut	Partially severed
Sternohyoid, omohyoid	Soft tissue haemorrhage	Not detected
Case 3		
Anterior L 4th–7th ribs	Fractures (left 4th–7th) with limited haemorrhage, fibrin deposition, osteocyte necrosis, early limited tissue response	Subtle fractures with sharp edges (6th + 7th) *Subtle fracture with sharp edges (4th + 5th)*
Rib heads R 4th, 5th, 8th and L 7th+8th	Deep osteocartilagenous fractures with fibrin deposition, subtle and patchy osteocyte necrosis	*Subtle fractures with sharp edges*
Rib head R 10th	Deep osteocartilagenous fractures with fibrin deposition, subtle and patchy osteocyte necrosis	Not detected
Anterior R 4th rib	Cortical shaft fracture	*Cortical shaft fracture with sharp edges*

4.2. Second reading

The micro-CT scans were re-examined against the histology findings in order to determine the micro-CT appearance of the features only identified using histology.

4.2.1. Case 1

The hyoid fracture seen on the micro-CT scan was confirmed histologically, as well as the possible fracture of the left superior thyroid horn. The anomaly identified on micro-CT on the left lamina is likely to be due to natural ossification processes as no fracture was observed here histologically. The haematoma seen on the slides were still not seen on the scan images due to soft tissue imaging limitations. Comparison of the histology report with the scan data confirmed that the displacement of the left superior thyroid horn is indeed artefactual as it was not apparent on the scan.

4.2.2. Case 2

The fractures of the superior thyroid horns were clearly visualised with both methods. The haemorrhage seen in the histology slides was not identified in the micro-CT scans due to insufficient image contrast. The tracheal separation described initially was attributed to the post-mortem dissection process.

4.2.3. Case 3

All but one of the additional fractures observed on the histology slides had been noted as potential abnormalities in the original micro-CT scan images. They had not been interpreted as fractures due to the image analyst's limited medical training. Fig. 7 shows sections through the right 5th rib which were seen to include the fracture identified histologically. The only fracture that was not seen during re-examination was that on rib R10.

5. Discussion

Forensic wound analysis in general can benefit from micro-CT in several ways. The main aim of wound analysis is to understand the mechanisms that have caused an injury. Micro-CT not only enables the detection of subtle fractures, it also provides the possibility to visualise these in a sanitised manner for use in court which can improve the jury's understanding of pathological findings. Medical grade CT is frequently used in medico-legal death investigations and its benefits have been discussed by Jeffrey et al. [4] or Roberts et al. [6]. However, their resolution has been found insufficient to image small samples or subtle injuries as demonstrated in studies by Robson Brown et al. [27] and Fais et al. [28] who present a case of a blunt force skull injury and a laryngeal fracture respectively which had been missed on standard post-mortem CT.

The fact that many of the fractures on the ribcage were not initially interpreted as such on the CT images raises the question of the image analyst's training. In the present study, images were interpreted by a researcher with a background in forensic anthropology and experience with examining micro-CT scans of skeletal elements but no formal radiological or medical training. Further inter-observer studies with participants from different professional backgrounds are therefore required.

The limitations of micro-CT highlighted in this study show that the technology will not replace or supersede physical histology techniques in forensic applications. However, comparing histology slides to the micro-CT images enables the histopathologist to determine whether a particular area of damage is real or a processing artefact, enabled by the non-destructive nature of the μCT scan which was demonstrated in case 1. The isotropic voxels of the micro-CT data allow virtual sectioning in any desired plane which, if used systematically, has the potential to maximise injury detection. This flexibility is frequently cited as one of the main advantages of using micro-CT for injury detection [29,30]. The 3D model created from the scan is particularly important as histology only provides a two-dimensional picture of a sample at a particular pre-selected location. Even slice sequences cannot fully compensate for this lack of three-dimensionality. Slices are taken at intervals of several millimetres which introduces the risk of missing small, subtle trauma. Having a three-dimensional image of the sample could assist the histologist planning their procedure to gather a maximum of information, thus improving the quality of forensic evidence. Histologists currently rely on macroscopic inspection and 2D radiographs to determine the orientation and location of their slices. Injuries which are invisible macroscopically due to the lack of haemorrhaging or displacement might evade detection. Fig. 4 demonstrates this for case 2 as the radiograph only shows one of the fractures. However, the resolution achieved by histology allows the examination at a cell level which is not possible on the CT data, at least not at the resolution achieved when scanning such large samples (the ribcage occupied the maximum dimensions possible in the CT scanner). Studying the cells is crucial as it is here where vital tissue reactions can be observed enabling the dating of the injuries which is a central aspect in medico-legal death investigations [31,32].

Few studies have employed micro-CT in forensic investigations [28,33,34] and comparisons of micro-CT and the current gold standard histology are even rarer [17,18]. This needs to be addressed as new technologies or methods can only be admitted as evidence if they have been tested and shown to be reliable. In the United Kingdom, these requirements have been set out under the new Criminal Procedure Rule 19.4 and the Criminal Practice Direction 19A.5 and 19A.6 [35]. The Forensic Science Regulator (FSR) is responsible for ensuring that forensic scientists adhere to quality standards which includes the use of validated methods.

Fig. 7. Micro-CT sections through the posterior fracture on the right 5th rib of case 3 (arrows). Re-examination of the scan after reading the histology report revealed the fracture which had previously been dismissed due to its subtlety. See Fig. 5 for the histology sections.

Validation is usually done using simulated cases, the use of actual case work material in validation studies is only acceptable if the new method is used for "general improvement" of the currently existing technique [36] which is the case in the present study which justifies the use of case material for validation. The current study presents three different samples but more studies would be recommended using a wider range of materials and a larger sample size in order to be able to make a rigorous assessment of the proposed method's limitations and potential error rates.

6. Conclusion

This study has demonstrated the potential of using micro-CT as a complimentary method to histology, the current gold standard for forensic injury assessment. By using three different post-mortem tissue samples it became apparent that μCT is a powerful method for detecting skeletal injuries but has limited use for unstained soft tissue. While it adds benefit to the existing method such as three-dimensional visualisation for planning and a non-invasive "as is" record of the sample, it currently cannot replace histology for soft tissue injuries or precise trauma ageing. There is an overall good correlation between the two methods but more research is recommended in order to make a full assessment of the novel method's strengths and weaknesses in compliance with current regulations for scientific evidence in the CJS.

Declaration of conflict of interest

None

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