Abstract

In August 2012 a skeleton was excavated in Leicester that was subsequently identified as that of Richard III, the last King of England to die in battle. In addition to a traditional full osteological analysis, an independent osteological assessment was undertaken using post-mortem computed tomography (PMCT). This publication presents the methods that were used for the PMCT examination of the skeleton, the results achieved and a comparison to the traditional osteological results. The results from the PMCT examination are comparable to those achieved from the osteological examination, but were carried out remotely, with no contact with the remains. This system is therefore extremely beneficial when dealing with fragile remains, particularly those of great historic significance.

Introduction

In August 2012 a skeleton was excavated in Leicester that was subsequently identified as that of Richard III, the last King of England to die in battle. The identification was achieved using primary, secondary and tertiary identifiers [1], including a DNA match with modern maternal-line relatives [2], archaeological evidence of the burial structure and location, and osteological analysis of the skeleton, respectively.

As part of the identification process a traditional osteological analysis of the entire skeletal remains was undertaken. In addition to this an independent osteological assessment was undertaken using post-mortem computed tomography (PMCT), blinded to the results of the traditional analyses. At the time of data analysis the suspected identity of the skeleton was also unknown. We present the method used for the PMCT assessment, the results achieved and a comparison to the traditional osteological assessment.

Materials and methods

Osteological analysis

Standard osteological methods were used, by direct observations of the skeletal remains, by a certified osteologist. Age was determined from the auricular surface of the ilium [3], the pubic symphysis [4], fusion of the medial clavicular epiphysis [5] and the development of the dentition. Osteological sex was determined from the pelvis, following the criteria set out by Phenice [6] and Brickley, 2004; and skull, from cranial characteristics described by Buikstra and Ubelaker [7] and characteristics of the mandible (relevant to British skeletal populations) in line with Brickley [8]. Metric analysis was also performed using the calculations denoted by Buikstra and Ubelaker [7]. Stature calculations were made using Trotter and Gleser's [9] recommendations for white males. Non-metric traits were recorded following the guidelines of Brothwell and Zakrzewski [10].

Post-mortem computed tomography

The PMCT image data were reviewed by an anthropologist experienced in PMCT, independent to the later known identification of the skeleton and to the biological profile determined by the traditional, dry bone osteological examination. The complete skeleton underwent PMCT examination. During the first scanning session, the bones were laid out in anatomical position. All bones were scanned with an Aquilion 64-slice scanner (Toshiba, Zoetermeer, Netherlands; 100

and 135 kVp; 60 mA; thickness 0·5 mm; matrix 512×512), reconstructed to 0·5 mm or 1 mm. After analysis of this scan, the bones were scanned again on a specially designed polystyrene mould to hold the bones in a truer anatomical position (Figure 1). The limbs, pelvis, spine, and head were scanned separately (100 kVp; 40 mA; reconstructed to 0·5 mm). The anonymized DICOM images were then transferred to an Apple Mac Pro workstation, and the radiograph data was processed for analysis using OsiriX 3D imaging software (version 3.7.1; distributed as open-source software under the GNU licensing scheme at the following Web site: http://homepage.mac.com/rossetantoine/osirix. Pixie: Switzerland).

Figure 1. Specially designed polystyrene mould to hold the bones in a truer anatomical position

A 'minimum data set' biological profiling form was constructed as described by Brough et al. 2014 [11]. As the remains in question were fully skeletonised (i.e. had no soft tissue remaining), and were not complete, the form was modified accordingly. The 3D skeletal view of the whole body was used to identify anti-, peri- and post mortem skeletal trauma (discussed in more detail in following sections), and congenital or acquired bone disease, for example scoliosis. The second page of the form included (where available), 7 views detailing the complete morphology of the skull; all the long bones and their measurements; a clear view of each joint of the shoulder, elbow, hip and knee; an isolated view of the hand, foot, pelvis and rib ends; the spine, sacrum and axis; and finally, dental orthopantomograms (OPT) reconstructions. The final minimum data-set form for the remains in question is illustrated in Figure 2 and Figure 3.

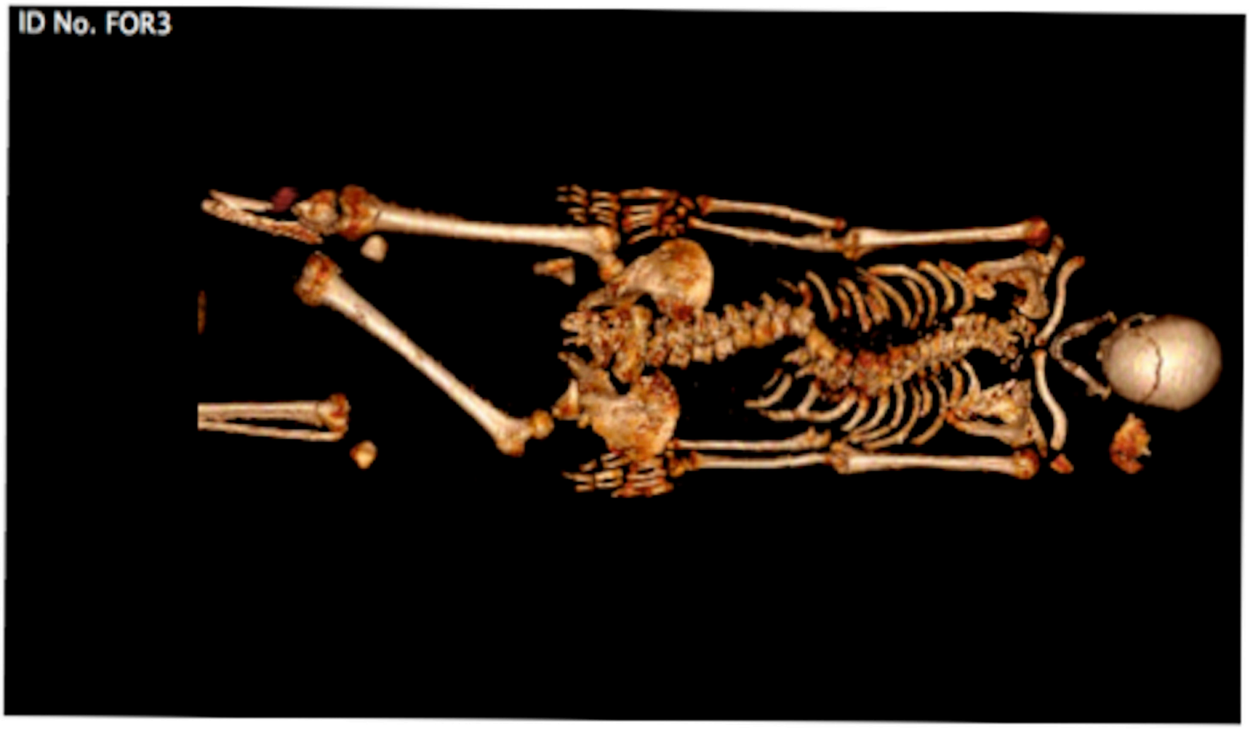

Figure 2: Minimum data set anthropological reporting form: Page 1.

Figure 3: Minimal data set anthropological reporting form: Page 2.

A total of 12 peri-mortem injuries were also identified on the minimum-data set form, nine to the skull and three to the postcranial skeleton. This prompted a request for supplementary images, which were subsequently provided (from the same/original dataset), for a more comprehensive final anthropological report (Figure 4).

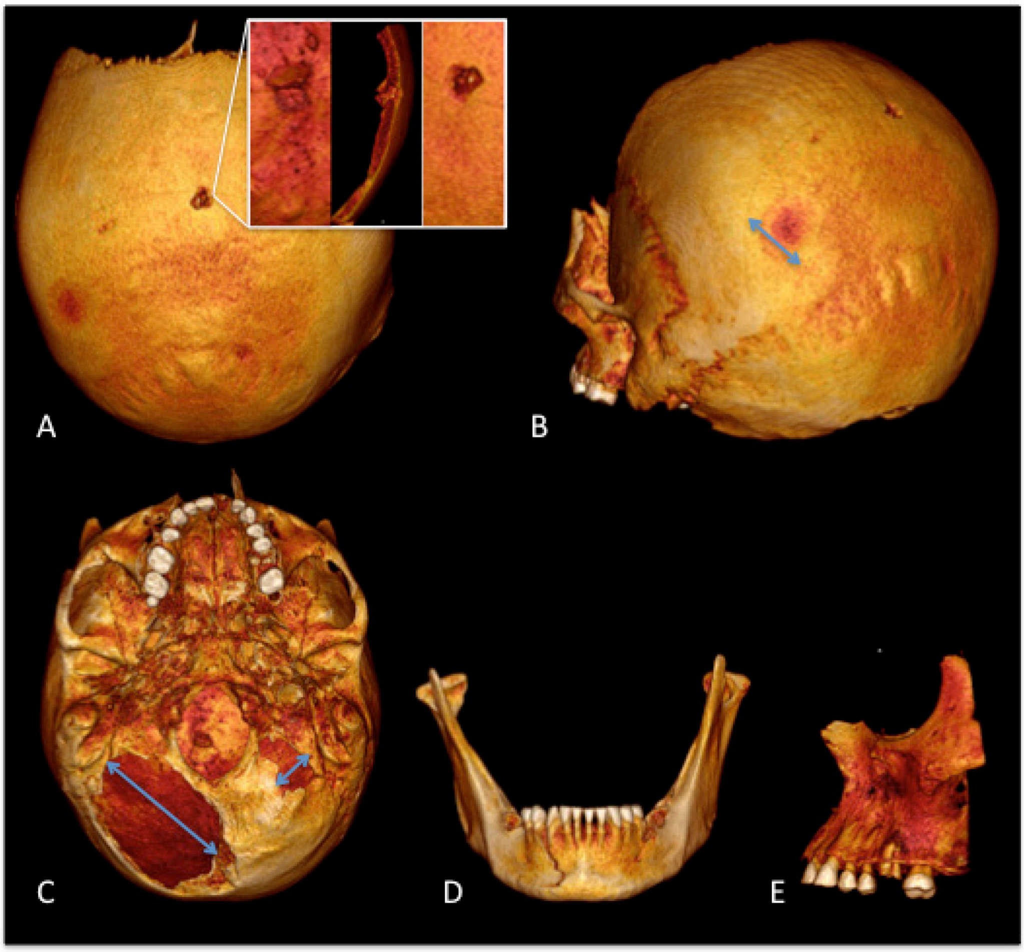

Figure 4: Additional images supplied on request, for trauma analysis. A) Keyhole trauma, B) superficial grazing injuries (red arrow), C) Bone loss to occipital region (arrows), D) Mandible trauma, E) Canine fracture and damage to the maxilla.

Results and Discussion

<u>Skeletal Preservation/completeness</u>

The skeleton was generally well preserved and largely complete (Figure 2). The distal tibiae and right fibula showed post-mortem breaks in the distal shafts, with the distal ends missing. The feet were missing, as was the left fibula, as discussed in a separate publication [12]. The right trapezium, trapezoid and pisiform bones were missing, as were one intermediate phalanx and nine distal phalanges.

There were two sites of damage caused during unearthing and excavation. The left tibia was crushed and a blow to the left side of the skull caused minor damage to the area of the coronal suture.

Sex

Using the methods discussed in this section, the skeleton was found to be male.

The majority of age and stature determination methods are sex-specific and therefore sex determination is normally the first step of the biological identification process. The two main morphological differences between male and female skeletons are; firstly, female bones are typically less robust and smaller than male bones, a feature which is particularly evident in the skull and secondly, size and shape differences of the pelvis reflect the biomechanical adaptations of the female pelvis to not only tolerate the compressive loading of locomotion, but also provide the expansibility and protective architecture required by late gestation and the birthing process [13].

Sex can be estimated using anthroposcopic traits or by metric methods. As this investigation was being conducted using only the PMCT minimum data form, which does not contain a detailed metric assessment of the skull or pelvis, only anthroposcopic traits were used in this investigation.

Sex could not be ascertained from the sacrum or pelvis, as there was no clear image of the sacrum. Also, because the pelvis had been manually reconstructed before scanning, the ventral arc (the raised ridge of bone that curves from the superior-medial corner of the anterior pubis, down and laterally, to where it merges into the inferior part of this bone) and ischiopubic ramus (the structure composed by the lower part of the pubis and ischium) were not clearly visible on the PMCT images, so could not be assessed.

Some of the characteristics of the skull that are helpful for estimating sex vary among ancestral groups. Therefore, the skull is generally considered secondary to the pelvis with regard to evidentiary power [14]. However, for completeness the skull was also assessed in this investigation. Table 2 summarizes the visual traits described by Krogman [15] and France [16], by which males are distinguished from females. Likewise, Buikstra and Ubelaker [7] presented drawings of five different variations of these characteristics, to help illustrate the transition from female to male. If a skull was highly consistent of category 1 traits the individual would be classified as female, whereas highly consistent of category 5 traits would represent a male individual. The skull in this investigation was consistent with category 4 or 5 for all traits, and would therefore be classified as male according to this system.

Table 1. Characteristics of male and female skulls*

Traits	Males	Females
Size	Large and rugged	Small and smooth
Mastoid	Large, projecting	Small, non-projecting
Browridges	Large	Small, none
Frontal	Slanted	High, rounded
Nuchal area	Rugged with hook	Smooth, hook uncommon
Supraorbital margin	Rounded	Sharp
Chin	Broad	Pointed

*Summarized from Krogman [15], and France [16].

Age

From an initial assessment of the PMCT images it was evident that all the dentition were fully erupted and therefore the remains were over 21 years of age. After the dentition is fully emerged and growth has ceased, several structures in the skeleton undergo changes over time that follow a rough timetable and can be used as an indication of age. Although these alterations are much more subtle and can be difficult to assess, by comparing these structures in a set of remains with accepted schedules of these changes provides a rough indication of adult age at death.

Four osteological features show clear stages of change during adulthood: pubic symphysis, auricular surfaces, sternal rib ends, and cranial sutures. The age range produced by the ribs is wider than that for pubic face therefore this method is generally considered less accurate than other techniques. The pubic symphyseal face indicated that the remains were 30-34 years of age (Figure 5). The pubic symphysis changes from an area of fine-grained bone covered with ridges to a flat area with porous, rough and otherwise 'old looking' bone. Similar transformations occur on the auricular surface of the ilium and on the sternal end of the ribs; these areas are originally composed of smooth bone that eventually becomes pitted with osteophytic development. The fourth structure that changes, the cranial sutures, close with age until they fuse and may eventually obliterate.

Figure 5. Pubic symphyseal face. Frontal view, illustrating the features required for a comprehensive evaluation.

The cranial sutures were scored between 0 (no closure) to 3 (complete obliteration). The composite score of these sites was then calculated. The composite score relates to a stage, which is then used to estimate an age [17]. All of the suture sites were visible on the PMCT images (Figure 6). However, a few were difficult to interoperate because of the image resolutions. The composite score for the vault was 6, as all the sutures were consistent with the description of partial closure. The composite score fore the lateral-anterior sutures was 5, three sites were consistent with partial closure and one was consistent with the description for significant closure. This gave an age range of 25-45 years (Vault); and 32-45 years (lateral-anterior). Therefore, the overall age estimation range from the cranial sutures was 25-45 years of age. This range is extremely wide and although it conforms with the pubic symphyseal face and rib end morphology age estimations, cranial suture age estimation is generally regarded as a less accurate. It was conducted in this investigation for completeness and to demonstrate that a full anthropological assessment could be carried out. However, the age range referred to in the final summary will be that made based on the pubic symphysis.

Figure 6. Cranial Suture closures

Ethnicity

The visual identification of morphological variations between different ancestral groups is the main method for assessing ancestry in adult remains. Krogman [17] listed the basic features of the cranium by which the three primary ancestral groups in the United States, White, Black and Asian, can be distinguished. A summary of these features, with additions from Brues [18] and Rhine [19], is presented in table 3. Using this table, the images included in the PMCT biological profiling form display Anthroposcopic features that are highly consistent with those diagnostic of a white individual.

Table 2. Anthroposcopic Characteristics of the skull of the three primary ancestral groups, as referred to in the majority of publications.

Structure	Whites	Blacks	Asians
Nose			
Root	High, narrow	Low, rounded	Low, ridged
Bridge	High	Low	Low
Spine	Pronounced	Small	Small
Lower border	Sharp (sill)	Guttered	Flat, sharp
Width	Narrow	Wide	Medium
Face			
Profile	Straight	Projecting	Intermediate
Shape	Narrow	Narrow	Wide
Eye orbits	Angular	Rectangular	Rounded
Lower eye border	Receding	Receding	Projecting

Vault			
Browridges	Heavy	Small	Small
Muscle marks	Rugged	Smooth	Smooth
Vault sutures	Simple	Simple	Complex
Postbregma	Straight	Depressed	Straight
Jaws and teeth			
Jaws	Small	Large	Large
Palatal shape	Parabolic	Hyperbolic	Elliptical
Upper incisors	Spatulate	Spatulate	Shoveled

* Combined from information in Krogman [15], Brues [18], Rhine [19].

Stature

The most accurate calculations will be obtained from undamaged bones of known sex and ethnicity, as stature is both sex and race dependent. The PMCT analysis of sex and ethnicity estimated that the remains are typical of those belonging to a white male. Therefore, these estimations were applied to the stature calculations to yield more accurate results. There was some taphonomic damage to the vertebrae and pelvis and both fibula and tibias were fragmented and only partially recovered; as a result, full skeletal methods of stature estimation could not be used in this examination. Although slightly less accurate than full skeletal techniques, methods using the long limb bones are arguably the most regularly used techniques for determining stature. The humerus, radius, ulna and femur were measured according to the standard measurements [7] (Figure 7). Both the right and left tibia and fibula were damaged in this investigation so could not contribute to the long bone stature estimation. The most reliable measurement available in this instance in the left femur. Using the standard formula for white males this indicated a stature in life of 1.74 ± 3.27 m, or around 5 foot 8 inches.

Figure 7. Long bone measurements

<u>Trauma</u>

This exercise presented an ideal opportunity to test the logistics of using a minimum data-set anthropological reporting form in a real case. As stated previously, the form provides a minimum standard of osteological reporting. However, if further images are required for a more comprehensive analysis of a particular region, the form creator can upon request produce additional supplementary images as specified by the form examiner. In this particular case, upon initial inspection a number of areas were identified that required a more in-depth examination. These included; areas of anti-, peri-, and post-mortem trauma that could have potentially been injuries procured during battle.

Figure 4 illustrates the additional images that were provided upon request, for a more comprehensive analysis of the regions/features of interest on the skull. The injuries detected from the PMCT images were all consistent with the injuries recorded during the dry bone examination. PMCT measurements were also all in agreement with those recorded on the dry bone. Importantly, PMCT identified two additional areas of trauma that were overlooked during the dry bone examination. However, PMCT failed to detect a sharp force tool mark of the right tenth rib-even when re-examined. The images were re-examined by both the anthropologist and a certified radiologist and on both the axial images and the 3D image reconstructions. It is possible that limitations related to resolution and slice thickness of the image lead to this extremely small injury being missed.

Summary

Osteological analysis results

The results calculated during the traditional osteological assessment were as follows:

- **Sex**: Male; from pelvis and skull morphology.
- **Ethnicity**: Was not assessed.
- **Age**: 22.2-38 yrs; from the schedule of changes in the pubic symphysis, auricular surface, and dental eruption.
- **Stature**: 1.74 m (5 foot 8.5 inches); from the left femur measurement applied to the stature reconstruction formulas for White American skeletons with European ancestry.

PMCT results

The biological profile produced from the PMCT investigation was as follows:

- **Sex**: Male; from pelvis and skull morphology.
- **Ethnicity**: Caucasian; from anthroposcopic traits of the cranium.
- **Age**: 30-34 yrs; from schedule of changes in the pubic symphysis, sternal rib ends, cranial sutures and dental eruption.
- **Stature**: 1.73 m (5 foot 8 inches); from the humerus, radius, ulna and femur were measurements applied to the stature reconstruction formulas given by Trotter and Jantz.

Table 3. Summary of results

	Osteological examination		**PMCT examination**	
	Result	Methods	Result	Method
Sex	Male	• Phenice [6] • Walker [20]	Male	• Krogman [15] • Buikstra and Ubelaker [7]
Age	22.2-38 yrs	• Brooks and Suchey's [4] • Buckberry and Chamberlain [3] • Ubelaker [21]	30-34 yrs	• Meindle et al. [22] • Krogman [15] • Iscan et al. [23,24] • Ubelaker [21] • Meindl and Lovejoy [17] • Ubelaker and Buikstra [7]
Stature	5 foot 8.5 inches	• Trotter and Gleser [9]	5 foot 8 inches	• Trotter [25] • Jantz [26].
Ethnicity	Was not assessed		Caucasian	• Krogman [15] • Brues [17] • Rhine [18].

The PMCT results were consistent with those calculated during the traditional osteological assessment.

Using the PMCT image data applied to the recording form it was evident that the skeleton was that of an adult male, in his late 20s to mid 30s with a gracile build and a severe scoliosis of the thoracic spine. This was consistent with the reports of Richard III age at death (33yrs) and physical description of slender build and raised right shoulder.

Limitations

The osteologist looking at the dry bone samples and the anthropologist looking at the PMCT images used different methods. This demonstrates the underlying issue that anthropologic evaluation of skeletal remains is subject to bias in function of the methods used. In this investigation the methods were not controlled, as it was not originally set up as a research project to compare the use of the same methods. By applying different methods, this investigation highlighted some interesting points for discussion. For example, the auricular surface of ileum is generally considered a reliable in indicator of age. However, the pelvis of the remains had been reconstructed before PMCT scanning and was imaged as a full assemblage with the sacrum included. The auricular surface of the ilium was therefore obscured in the final images and even with 3D image analysis software; the sacrum could not be assessed independently without altering the auricular surface. This could be avoided by scanning the bones separately. In intact cadavers this is not a problem and the articular surfaces are separated by cartilage and other soft tissues allowing them to be virtually dissected using image analysis software. As a result this area could not be properly assessed during the PMCT examination and the ribs were evaluated instead. This situation parallels the difficulties that might be experienced when dealing with forensic cases of articulated, or partially decomposed bodies.

Conclusion

The PMCT method used is shown to be comparable to traditional osteological techniques and allows for remote virtual anthropology for identification purposes [27]. As the skeleton was of great historical value great care had to be taken during handling. This caused a number of issues when measuring the dry skull. Unlike most forensic cases, where the skeletal elements could be reconstructed using resin or glue to make measurements easier to obtain, in this situation the skull had to be carefully held together by a team of practitioners while the measurements were recorded. This is time consuming and was resolved using PMCT. The results of the two investigations were comparable. As different methods were used a statistical analysis of the results for a more comprehensive comparison could not be achieved. Some areas of the skeleton were obstructed on PMCT, such as the auricular surface of ileum. This was therefore more easily assessed on the dry bone specimens. In future PMCT scans for osteological analysis will be performed with separation of key bones. However, in spite of viewing restrictions, the PMCT estimations were within the same range as the osteological ones. The PMCT analysis was quicker and could be carried out without any damage to the remains. This is an important advantage, particularly when dealing with ancient remains. In addition, the PMCT data could be used to create 3D prints of the bones, which could be used for education, research or display in several locations around the world. In terms of a forensic investigation, PMCT could therefore be used to estimate the biological profile of an individual with minimal disruption to evidence.

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