Manuscript Details

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Title	An investigative strategy for assessment of injuries in forensic anthropology
Article type	Short Communication

Abstract

In a forensic context, identification of skeletal lesions' and traumas' timing may be of fundamental relevance to understand the events related to the life and death of an individual. In this study, we propose a new evaluation form to facilitate the detection of traumas and interpret them as ante-, peri- or post-mortem injuries. We describe the use of this form with the analysis of two skeletonized individuals. Bone injuries on their skeletons were caused by diverse sharp weapons and differed for the timing they have occurred, as revealed by macroscopic, radiographic and microscopic assays. Thanks to its completeness and user-friendly approach, the evaluation form here proposed may greatly facilitate the analysis and interpretation of lesions found on skeletons under forensic investigation.

Keywords	forensic science; forensic anthropology; skeleton; lesion timing.
Taxonomy	Investigation of Injury, Trauma, Inflicted Injury
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Suggested reviewers	Jan Novacek, Margherita Neri

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Dear Editor,

I am pleased to propose this new manuscript entitled "*An investigative strategy for assessment of injuries in forensic anthropology"* (by Filippo Scianò, Barbara Bramanti, Vanessa Samantha Manzon, Emanuela Gualdi-Russo) to be considered for publication as a Short Communication in your journal – Legal Medicine

In this manuscript, we propose a new lesions' identification form for assessing type and time of trauma in dry bones – an often difficult appraisal in forensic contexts. We have employed the form on two human skeletons that showed interesting lesions in different osseous districts. The lesions of the first individual (Case 1) involved the skull, while those of the second individual (Case 2) involved both skull and spine. We describe in details the different injuries, investigated using a multifaceted approach, which involves anthropological, taphonomic, radiological and microscopic analyses. The outcomes are interesting, also because they demonstrate that it is possible to reconstruct aspects of the biological and taphonomic life of the two individuals.

In our opinion, the proposed form may highly simplify the assessment of skeletal injuries in the forensic context, thus effectively contribute to obtain retrospective diagnosis of ante-, peri- and post-mortem lesions from dry-bones.

I sincerely hope our contribution meets the interests and standards of your prestigious journal. All coauthors agree with the submission of the manuscript, while the research constitutes our intellectual property. The authors declare no competing financial interests. This work is an original contribute.

Thank you for your consideration,

Kind regards,

Barbara Bramanti
Corresponding Author

Dear Editor, Dear Reviewers,

Please, find below a table with our answers to your comments and the indications of change done in text, table and picture.
We would like to thank You for your very useful suggestions that we think have improved the quality of the manuscript.
We hope this new version of the manuscript will be appreciated and accepted for publication.

Best Regards,
The Corresponding Author

Decision Letter - Revise: 16 July 2019
Ref: LEGMED_2019_204

Comments of Editors and Reviewers	**Answers of the Authors:**
Reviewer 1: The paper provides a comprehensive and detailed anthropological study on the interpretation of skeletal defects. Two cases studies dealing with multiple lesions of difficult interpretation are reported. They have been investigated in a remarkable methodological way by macroscopic, radiographic and histological assays. Findings are clearly illustrated with figures of very good quality. A valuable trauma evaluation form is proposed. It can represent a useful reference in the interpretation of skeletal defects not only in archaeological settings but also in forensic cases. The overall quality of the article is very good. In my opinion, the present study is of a standard suitable for publication after minor changes. 1) I invite the Authors to spend some few words on the signs of osteoblastic activity found on the skull of individual US 114 as depicted in the sub-figures d) and e) of Figure 2. 2) Unfortunately the Howship's lacunae are not so clear and a magnification of these features could be better appreciated by the reader. 3) Please, check reference final list and, in particular, reference number 7, 8, 11, 12, 19, 36, 44.	Thank You, all your proposals have been accepted: We added a sentence about the osteoblastic activity detected on the skull of individual US 114, taking in consideration the signs of bone remodeling, macroscopic and microscopic evidences (Lines 65-69) A new microscopic picture with higher magnification (400x) was taken and included in Fig. 2e. The reference list was checked and revised using the reference style for Mendeley Desktop according to the Guide for Authors. Nevertheless, we have found only four citations that needed revision (7, 8, 36, 44).

They all need to be revised and cited following the reference style requested by the Guide for Authors	
-Reviewer 2 In dry bone, the definition of “lesion” is not clear . Maybe “injury” should be a better option. In fact, the authors underline in the Abstract that they “propose a new evaluation form to facilitate the detection of traumas and interpret them as am or pm injuries”. Designation “lesions” is not correct in this perspective. In the Discussion, the authors point out that "forensic anthropologists assist anatomo-pathologists". In a forensic context, forensic anthropologists assist forensic pathologists, not anatomo-pathologists. Designation “anatomo-pathologists” is not correct.	Thank You, all your proposals have been accepted: The terms ‘lesion/s’ were replaced with ‘injury/ies’ everywhere in the manuscript, included the title description of Fig. 1. In the discussion, particular attention was given by replacing the inappropriate terminology with the most appropriate one suggested by You.
Other comments: Data in Brief (Optional) Legal Medicine is now partnered with Data in Brief You are invited to submit a data article to Data in Brief alongside your research article. A data article is an attractive alternative to supplementary material and publishing in Data in Brief ensures your data, and the metadata to understand it, is independently reviewed, formatted, indexed, given a DOI and made publicly available online. To submit your data article to Data in Brief, please use the template available through the link below. When your research article is accepted, your Data in Brief article will then be transferred automatically.	We have no data for Data in Brief.

Highlights

- Proposal of a new evaluation form specific for skeletal injuries.
- Assessment of lesions' features with different approaches including macroscopic, microscopic and radiological analyses.
- Proposal of a reasoned strategy for forensic anthropological research to assess the timing of lesions in human dry bones.

An investigative strategy for assessment of injuries in forensic anthropology

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Abstract

In a forensic context, identification of skeletal injuries' and traumas' timing may be of fundamental relevance to understand the events related to the life and death of an individual. In this study, we propose a new evaluation form to facilitate the detection of traumas and interpret them as *ante*-, *peri*- or *post-mortem* injuries. We describe the use of this form with the analysis of two skeletonized individuals. Bone injuries on their skeletons were caused by diverse sharp weapons and differed for the timing they have occurred, as revealed by macroscopic, radiographic and microscopic assays. Thanks to its completeness and user-friendly approach, the evaluation form here proposed may greatly facilitate the analysis and interpretation of injuries found on skeletons under forensic investigation.

Keywords: forensic sciences, forensic anthropology, skeleton, lesion timing.

1. Introduction

Bone traumas detected on skeletons were described by Dirkmaat et al. (2008) as «a moment frozen in time which consistently contributes to understanding what had happened to the deceased individual». In forensic sciences, it is of particular importance to identify the type of injury inflicted and the weapon that caused it [1]. Accordingly, several different types of skeletal traumas can be distinguished as cutting wounds, blunt force traumas, sharp force traumas, chop wounds, stab wounds, ballistic wounds and several others [2–4]. Yet, another crucial aspect in the evaluation of skeletal traumas is to determine whether the injury occurred long before death (*ante-mortem*), at death or shortly before death (*peri-mortem*), or long after death (*post-mortem*). Peri-mortem injuries may help in defining the cause of death, whereas ante-mortem injuries detected on the skeleton may be useful for the identification of the victim. Finally, post-mortem injuries can provide indications about the fate of the body between death and discovery.

To help in the distinction of ante-, peri- and post-mortem skeletal injuries, we have generated a new user-friendly diagnostic form, which resumes the observations of several scholars [2,5–14] (Fig. 1). The analyses of the injuries are mainly based on morphological features detectable macroscopically, yet we suggest to integrating the information with radiological, microscopic and chemical techniques [4,15,16], when necessary. Hereafter, we show the employment of the evaluation form on two case-studies with multiple injuries, thus of difficult interpretation.

2. Timing and interpretation of skeletal injuries

Injuries occurred during life are characterized by bone remodelling due to the ability of the bone tissue to regenerate [17], whereas injuries produced on the skeleton, i.e. on the dry bone (dry fracture), are identifiable on the basis of characteristic bone patterns due to the loss of tissue elasticity [13,18]. Indifferently if they are intentionally inflicted or due to taphonomic processes, post-mortem injuries show an irregular fragmentation of the bone, with sharp margins and a colour of the edge surfaces, which differs from the rest of the bone [17].

More difficult is to distinguish between injuries occurred shortly before or shortly after the death [15,18] since both kinds of *peri-mortem* injuries show patterns that depend on the elasticity and plasticity of the living tissue with smooth edges of the same colour as the nearby bone.

Taking into account the considerable literature that has been devoted to this topic in forensic anthropology [2,6,13,19–22] we have summarized the characteristics of ante-, peri- and post-mortem injuries in a specific evaluation form (Fig.1), which offers a synthetic description of each feature and specific references from the literature. To facilitate the identification and interpretation of traumas, we integrated the descriptions with pictures.

3. Case studies

We isolated two interesting cases of trauma found among 122 skeletons dating back to the 17th-19th centuries from the cemetery of the San Biagio church (Ravenna, Italy). Sex, age at death and stature of the skeletonized individuals were determined using standard anthropological methods [23–35]. Cranial and post-cranial macroscopic evidences of injuries were examined in depth with a magnifying glass and a light microscope, to reduce the risk of misinterpretation. Radiographic and histological techniques were also employed to reinforce the evidence. Analysis and interpretation of the injuries were carried out using the new evaluation form **(**Fig. 1) and reported in Table 1**.** Injuries' features of both cases are reported in Table 2.

3.1. Case 1: The individual US-114 was a 45-55 years old man, 169.5 ± 4.3 cm tall. A deep injury was detected on the right supraorbital margin of the frontal bone **(**Fig. 2a**)**. The injury appears fairly large with irregular edges and shape. The surface interested by the direct kerf is smooth with some parallel striations that form a 90° angle with the transverse plane. All around the hit area, there are evidence of bone resorption and remodeling (bone spicules and osteolytic reactions) [2,36–38] (Fig. 2 b,c,d,e). Microscopic signs of specific cell activity are presents, the lamellae appear smoother due to advanced osteoblastic activity, while the presence of several Howship's lacunae resulting from the osteoclastic activity are already visible (they occur usually 4 days after the trauma) [39–41], as well as the occurrence of absorption of some cortical bone tissue adjacent to the site of trauma. All

features suggest that the injury occurred *ante-mortem***.** In support of this hypothesis, we observed evidence of a periosteal reaction (*osteoperiostitis*) on the outer surface of the injury, while traces of a nonspecific infection (*osteitis*) are visible on the inner surface. Both evidence might be the consequence of the same injury [2,42]. Radiographic and histological analyses confirmed this interpretation and allowed us to identify the injury as an *ante-mortem* injury (Table 1). Another hypothetical consequence of this traumatic event was a severe displacement of the mandible, with the right condyle shifted from its natural joint point, along the zygomatic arch where it formed a new articulation (Fig. 2 f,g,h). Any evidence points to a survival of the individual to the severe injury.

3.2. Case 2: Individual US-118 was a 30-39 years old male, 162.6 ± 3.3 cm tall. Multiple traumas of the skull and spine are detectable on his skeleton (Fig. 3). The first is a small and deep cut-mark on the right mastoid process (Fig. 3a; Table 1). Macroscopic and microscopic analyses have shown that the edges of the walls were perfectly clean and clear and there was no presence of porosity around the cut mark; the color of the kerf was brighter than the rest of the mastoid surface. Apparently, the injury occurred post-mortem.

Other traumatic evidences are a series of sharp cut-marks at the level of the 10ththoracic vertebra (T-10). Among them, the most evident is on the upper-endplate, while two others are placed on the spinous process and on the right transverse process (Fig. 3 b,c,d,e,f). These last two injuries, probably stab wounds, were likely inflicted simultaneously since they seem to show the same trajectory of attack, inclined by 60° with reference to the longitudinal axis of the vertebrae. This set of stab injuries shows neither porosity nor osteogenic reaction and inflammation, nor osseous regenerative process or pathological reaction, as would be expected in case of an ante-mortem trauma [6,19]. The cut marks have a V-shaped cross section with the edges of the walls inflected towards the kerf; the uniform colour of the entire area around the cut marks suggests that the injuries were inflicted when the bone was still elastic [3,43–45]. The absence of bone remodeling and inflammatory reaction indicates that the individual died shortly after being stabbed (Table 1).

4. Discussion

The two case-studies presented highlight the distinction between injuries of the dry bone tissue by traditional anthropological methodologies, supplemented by radiographic and histological analyses, in order to define the timing and/or dynamics of events. The application of the new form was of considerable help in collecting the elements useful for the diagnosing of *ante-* vs *peri-*or *post-mortem* injuries.

In case 1, the sharp force trauma inflicted was presumably a chop wound; this type of injury is produced by heavy and cutting instruments like axes, machetes, adzes and some types of swords [4]. Considering the historical period and the geographic localization, a sword or a lumberjack/farmer axe [44] are the most likely weapons. Periosteal inflammation and bone remodeling, as well as macroscopic and microscopic evidence of healing, testify that the individual survived the trauma [2].

In case 2, the cut mark on the mastoid process was probably inflicted with a sharp-edged tool during the unearthing of the skeleton, since the absence of osteogenic reactions excludes an *ante-mortem* event, whereas surface features, shape and color exclude a trauma occurred during the individual's lifetime. Conversely, the vertebral injuries of the same individual were probably inflicted near the individual's death by a small and sharp-edged weapon (e.g. a knife or a dagger) according to the dimensions and morphology of the injuries [44]. As the width of kerf and the groove reflect the blade dimensions of the offending weapon [13], the reduced dimensions of the injuries, especially on the posterior surface of T-10, suggest that they were due to the thin thickness of a blade. Although the margins' introflexion and the elastic deformation of the bone surface - typical of *peri-mortem* trauma - are clearly visible, the complexity of this type of diagnosis on the spongy bone precludes the absolute certainty about the timing of injury. If he was stabbed in life, then he did not survive the attack. Although unlike, we cannot rule out that the *peri-mortem* injuries date back to a period immediately after the death of the individual, since the tissue components are the same as the living tissue (green bone) with responses indicating high elasticity and plasticity. Particular caution is required in the interpretation of this type of injuries in the forensic context.

Unlike forensic pathologists who generally rely on the entire body of the victim, forensic anthropologists cannot determine always the cause of death with absolute confidence [9,38,46]. Nevertheless, providing information on skeletal injuries, along with the biological profile (sex, age, stature, ethnic origin, as well as pathological injuries and congenital skeletal anomalies) of the victim, and knowing the taphonomic conditions, a forensic anthropologist may assist the forensic pathologist in formulating hypotheses and drawing conclusions , which may contribute to [3,47] or be decisive for the resolution of a case.

Therefore, as Kanetake et al (2008) [48] pointed out, when dealing with skeletal remains, the issues raised by the judicial authority should be addressed by a team of specialists, including forensic anthropologists.

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TABLE 1 - Assessment of the time of trauma occurrence in the examined case-studies

	Healing process	Bony responses	Case 1	Case 2	
			Skull	Skull	Post Cranium
Ante mortem	1. Formation of the hematoma.	End of bleeding	✓	-	-
	2. Cellular proliferation. Fracture is joined and visible on dry bone	Pathological reaction (periostitis). Sub-periosteal bone formation	✓	-	-
	3. Mineralization of osteoid. Visible radiologically.	Formation of the (osseous) callus made of woven bone	✓	-	-
	4. Consolidation of the woven bone	Primary and secondary lamellar bone formation.	✓	-	-
	5. Gradual remodeling of bone	Increase of density and strengthening along lines of mechanical stress.	✓	-	-
Peri mortem	Absence of healing processes	Edges of injuries irregular and shaped; hinging bone fragments. Cortical bone flakes along fracture margins. Plastic deformation.	-	-	✓
Post mortem	Absence of bony response or healing	Fracture margins colored of bright white, or lighter. Bone shatters into regular fragments.	-	✓	-

TABLE 2– Principal features of the injuries observed in the two cases

Case No.	Trauma number - per person	Trauma number - per bone	Location (side)	Shape	Size (cm)	Timing	Possible Etiology
1	1	1, skull	• Frontal, Supraorbital margin (R)	Semilunar, irregular	3.8 x 1.3	Ante-mortem	Heavy sharp weapon
2	4	1, skull	• mastoid process (R)	Straight, regular	1.3 x 1.1	Post-Mortem	Accidental, unknown
		3, T-10	• inferior surface of v. body	Straight, irregular	3.5 x 0.7/0.1	Peri-mortem	Stab
			• spinous process	Sharp, regular	0.3 x 0.3	Peri-mortem	Stab
			• transversal process (R)	Sharp, regular	0.6 x 0.1	Peri-mortem	Stab

Figure Legends

Fig. 1 - *Trauma evaluation form with illustration and description of the features of different injuries and healing processes.*

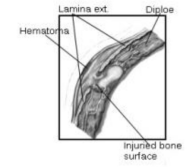
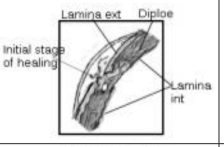
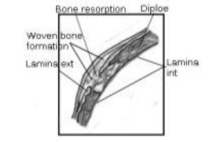
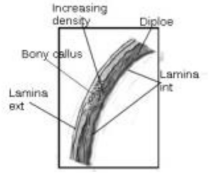
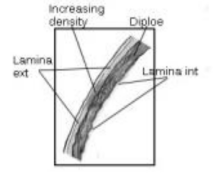
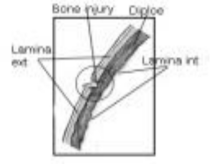
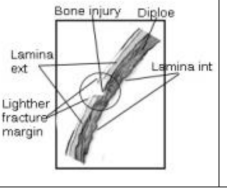
Fig. 2 - *Skull of individual US 114. a) Chop wound on the right supraorbital margin; b) Right orbital roof surface, with inflammatory process of the bone (white arrows); c) CT scan of the traumatized area, with different density districts (white arrows); d) Histological section: view through the light microscope (15 µm section in Masson's trichrome stain, Magnification 40x) of the right supraorbital margin, with cortical bone, remodeled after the injury (osteoblastic activity) on the entire surface (red arrows); e) Histological section of the right supraorbital margin: view through the light microscope (15 µm section in Masson's trichrome stain, Magnification 100x) of the traumatized area with inflammatory reaction on the outer surface (red arrows) and signs of osteoclastic activity (Howship's lacunae – green arrows; Magnification 400x); f) Mandibular malocclusion: dislocation of the temporo-mandibular joint; g) Creation of new articular facet on the inferior inner surface of the zygomatic arch (white arrow); h) Erosion and adaptation of the right mandibular condyle.*

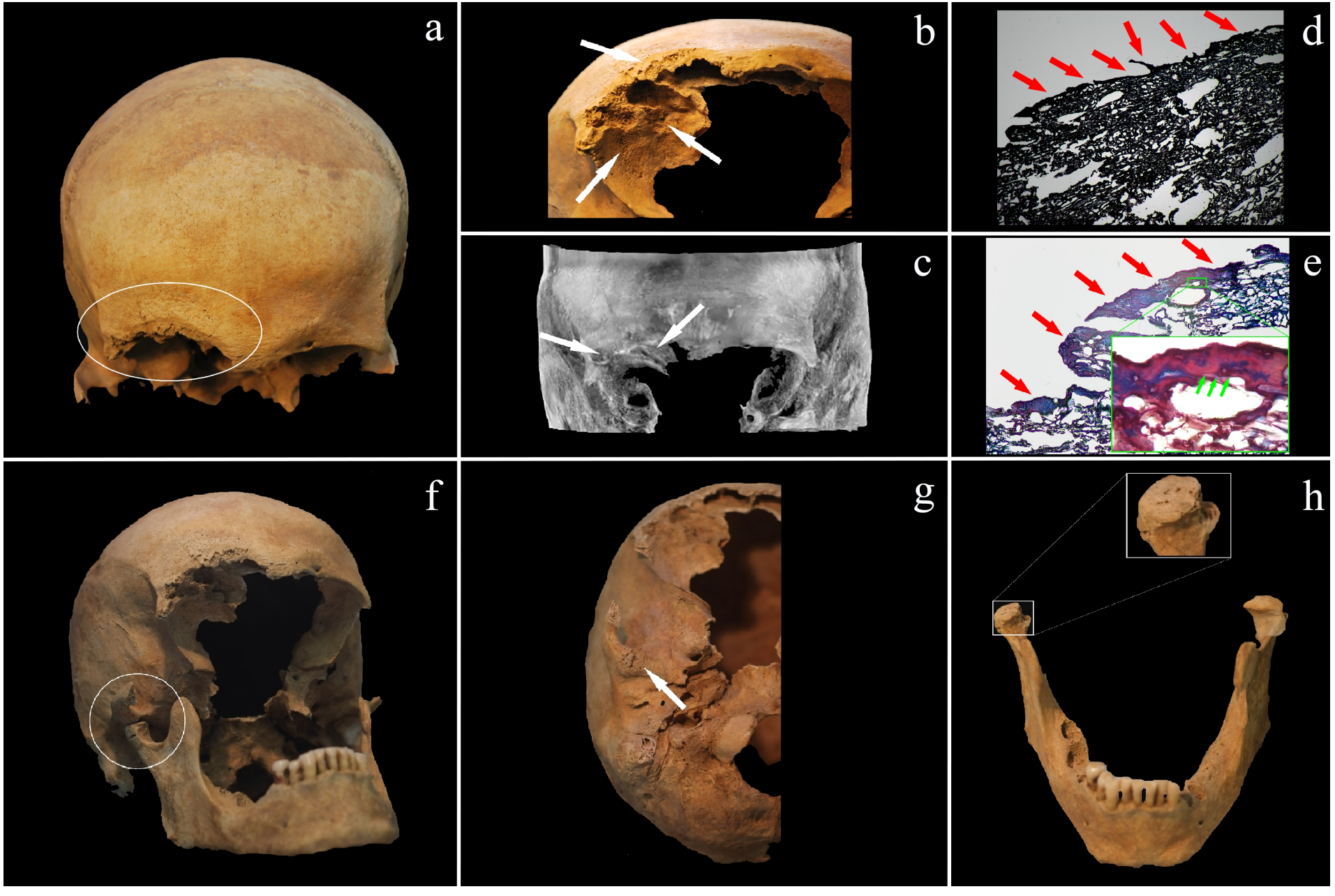
Fig. 3 - *Pictures of the mastoid process and the 10ththoracic vertebra of individual US 118. a) Detail: post mortem cut mark of the right mastoid process; b)Localized sharp trauma on the bottom of the vertebral body (square, a1); c) Detail: sharp force trauma at the light microscope (10x magnification); d) Cut marks localized on the spinous process (c1) and on the right transversal process (c2); e) Detail: sharp force trauma of the spinous process (light microscope, 10x magnification); f) Detail: sharp force trauma on the right transversal process (light microscope, 10x magnification).*

Fig. 1 – Trauma evaluation form: charactheristics of injuries and healing processes

Archaeological site_____ _____ **Individual**_____

Date_____ _____**Evaluator**_____

Time of trauma	**Healing process**	**Duration**	**Bony responses**		**Presence**
Ante mortem	1. Formation of hematoma. Injured bone starts to regenerate due to the arrest of the hemorrhage	Within 24 hours	End of bleeding. Formation of hematoma histologically determinable.		☐
	2. Cellular proliferation with deposition of osteoid tissue around each fragment by osteoblasts of periosteum and endosteum. Fracture is joined and visible on dry bones	Within 3 weeks	Osseous reaction. Presence of periostitis. Initial stage of healing		☐
	3. Mineralization of osteoid that works such as a split for the periosteal and endosteal surfaces. Visible also radiologically	From 3 up to 9 weeks	Bone resorption and consequent formation of the callus made of woven bone (*soft callus*)		☐
	4. Consolidation of the woven bone from callus precursor that results in a solidly united fracture area.	From few weeks to few months	Lamellar bone formation (*hard callus*)		☐
	5. Gradual remodeling of bone to its original form.	From 6 to 9 years	Increase of density and strengthening along lines of mechanical stress radiologically determinable		☐
Peri mortem	Occurring at or near the moment of death	Absence of bony response or healing	• Edges of injuries irregular and shaped; hinging bone fragments; • Cortical bone flakes along fracture margins; • Internal or/and external smoothed margins; • Radiating and concentric fractures; Staining maintained along fracture margins; • Plastic deformation.		☐
Post mortem	After death	Absence of bony response or healing	• Fracture margins colored of bright white, or lighter; • Bone shatters into regular fragments; • Possible taphonomic evidence (e.g. gnawing or carnivore puncture marks).		☐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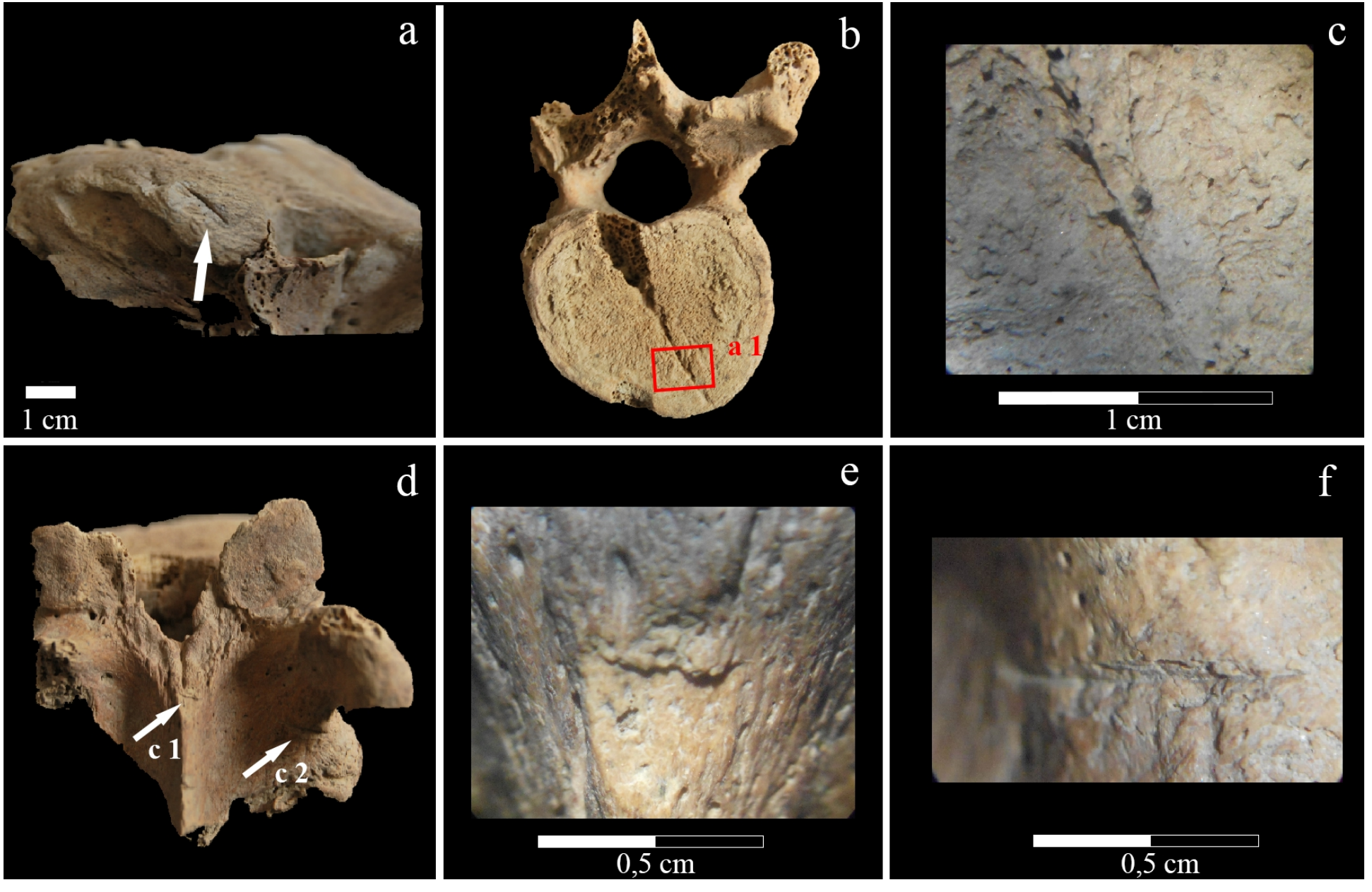
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