Title: Osteolytic lesions on the *os petrosum* of a Bronze Age individual from La Llana cave (Northern Spain) compatible with a possible case of otitis media. A multifaceted methodological approach.

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Abstract

Objective: This report aims to encourage the use of different methodological approaches for the most complete identification in paleopathology, and provides an example of an osteolytic lesion found on the intracraneal surface of a temporal bone from a Bronze Age individual at La Llana cave (Spain).

Materials: Cranial remains (fragmented *post-mortem*), from a 3,300 ± 25 BP (1631-1509 cal BC) adult male from La Llana cave (Spain) with significant, possibly pathological, erosion of the right and left temporal bones.

Methods: The cranium underwent macroscopic, microscopic and computed tomography scan examinations.

Results: *Tegmen tympani* of the right temporal bone has disappeared, while on the left temporal bone it is still present. Both *cochleae* are intact. The individual presents porotic lesions on the external surface of the mastoid process near the ear. Neither mastoiditis nor any other pathology has been identified.

Conclusions: The lesions are compatible with otitis media (atticitis) that eroded the bone, resulting in expansion of the infection into the meningeal layers.

Significance: By showing the relationship between middle ear morphology (CT-scans) and endocranial surface of the petrous bone and the microscopic and macroscopic characteristics of otitis media, the effects of the infection could be identified even on temporal bone fragments.

Limitations: The scarcity of systematically described and studied otitis-related lesions in archeological contexts for comparative purposes.

Suggestions for Further Research: The use of different approaches to diagnose otitis media in archeological skeletal collections is recommended to improve the knowledge of health status and lifestyle of past populations.

Keywords

Atticitis; Prehistory; Atlantic Bronze Age; Osteoclastic activity.

1. Introduction

Diagnosing diseases in archeological contexts is a challenging process since multiple pathological conditions can produce comparable lesions on bones, and even taphonomic factors can mimic some pathological conditions (Aufderheide et al., 1998; Buikstra et al., 2017; Klaus, 2017; Lovell and Grauer, 2018).

In this report, two osteolytic lesions found on the *eminentia arcuata* from a human cranium from La Llana cave (Asturias, Northern Spain), dating to the Early Bronze Age, were studied. Differential diagnosis utilized location, morphology and RX inspection of the lesions.

2. Materials

The human remains studied here came from La Llana cave in Asturias, Spain (Figure 1). The archaeological site was discovered in 1981 during archaeological survey (Pérez Suárez, 1982) and was later excavated by one of the authors (M. R. González Morales) between 1982-1985 (González Morales, 1995). The cave was formed during the karstification of the coastal platform and is located one kilometer from the current coastline. The cave has two entrances: a large entrance located in a deep doline at the northern end and a small rockshelter to the south (nowadays nearly obstructed by sediment). The archaeological excavation inside the cave was divided into two different areas: Sector A, where a human burial was documented lying on the surface, with part of the bones covered by a stalagmitic flowstone firmly concreting them to the cave floor; and Sector B, where a Mesolithic shell midden was found, divided into two layers (Stratigraphic Units 1 and 2). An initial taphonomic study of the human remains shows that some bones were still articulated (Figure 2), whereas other bones had been displaced due to the decomposition of flesh and a low-intensity water flow responsible for the calcrete layer covering the bones (González-Rabanal et al., 2018).

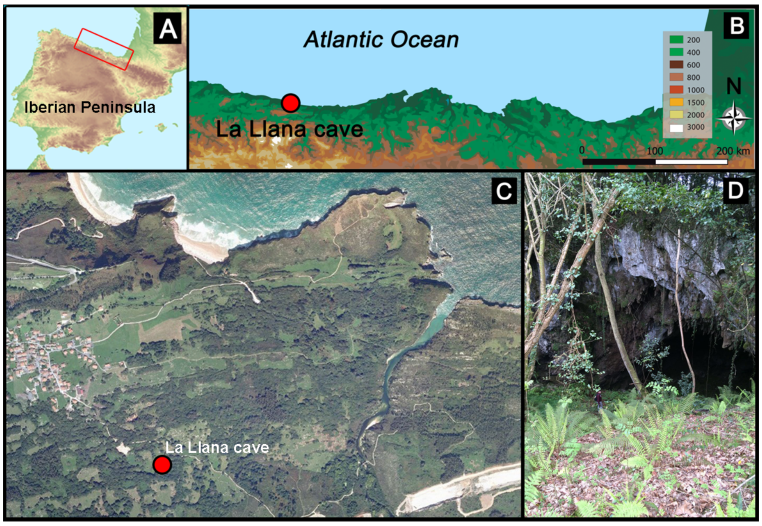

Figure 1: Geographical location: A. Position of the Cantabrian Region in northern Spain. B. Location of the La Llana cave within the Cantabrian Region. C. Position of the site in relation to the coastal platform and its proximity to the sea. D. Entrance of the cave.

The human bones were gently cleaned with a soft brush and water and photographed *in situ*. The remains that were less well adhered to the floor or in the clear risk of being damaged were recovered to prevent their destruction. This was the case for the cranium, of which the left part was already broken *post-mortem* and had sunken into the cranial vault. The skeleton belonged to an adult male with a stature of 165-168 cm (González-Rabanal et al., 2018), dating to the Middle Bronze Age (3300 ± 25 BP; 1631-1509 cal BC) (Vega Maeso, 2017) based on C-14 AMS dating of the remains at the Center for Applied Isotope Studies from the University of Georgia, USA (Sample ID: UGAMS-9083).

The endocranial surface of both temporal bones exhibited osteolytic modifications, which likely stem from a pathological origin as opposed to taphonomic alterations as outlined below. The only other antemortem modifications in the skeleton (both cranial and postcranial) compatible with paleopathologies are a small abscess exposing part of the root of the upper right first molar and a carious lesion affecting the crown and neck of the right upper second molar.

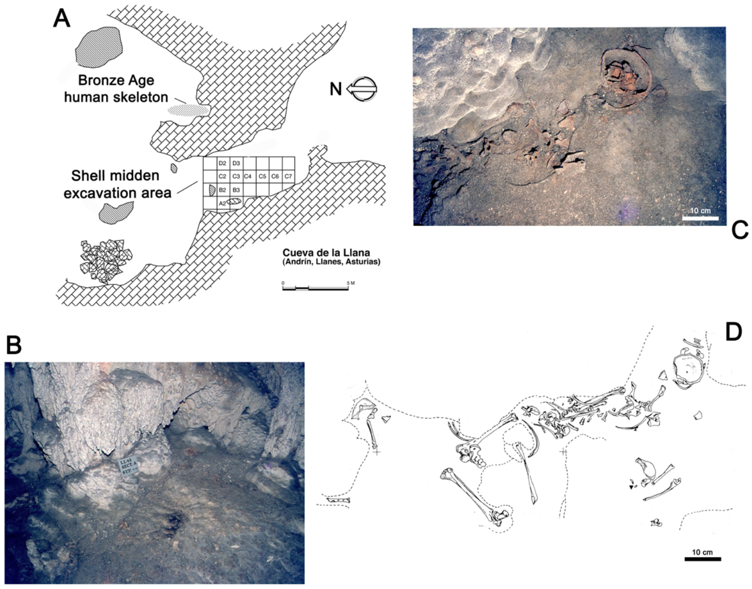

Figure 2: Position of the skeleton within the cave: A. Map of the cave system showing the excavation area. B. Stalagmitic flowstone where the human skeleton was found. C. Preservation state of the cranium when discovered. D. Spatial distribution of the human skeleton.

3. Methods

The temporal bones from the La Llana individual were inspected macroscopically under a binocular lens at 10-80X magnification. In order to differentiate bone changes as a consequence of post-depositional processes from those of paleopathological origin, taphonomic guidelines were used (Botella et al., 2000; Lyman, 2004; Fernández-Jalvo and Andrews, 2016). Additionally, the temporal bones were compared with the human reference collection at the International Institute of Prehistoric Research of Cantabria (IIIPC). This reference collection derives from 20th-century cemeteries within the Cantabria and Basque Country regions in Northern Spain, and contains individuals ranging from 0 months to 70+ years. Seventeen temporal bones displaying no skeletal pathological or other anomalous morphologies were used for comparison. Additionally, twelve archaeological temporal bones from the same period (Chalcolithic and Bronze Age) also recovered from the on the surface of cave floors and thus subjected to similar taphonomic conditions, were used to assess the possibility of postmortem change in this specimen. The comparative sites used were the burial caves of: Los Avellanos I and II (n=5) (Vega Maeso, 2017) curated at the Museum of Prehistory and Archaeology of Cantabria (MUPAC), La Castañera rockshelter (n=1) (Jones et al., 2019) and El Espinoso burial cave (n=6) (González-Rabanal et al., 2017), all currently curated at the IIIPC.

Observed bone changes to the La Llana cranium were first documented macroscopically and recorded with photographs. In order to analyze the histological surface of both lesions and to check for bone resorption or formation, a digital optical microscope up to 1000X was used. In addition, both temporal bones were scanned with a Resolution EVO Scan (GE Medical Systems) located at the Marqués de Valdecilla University Hospital (Santander, Spain). Medical computed tomography (CT) scans (120 kv, 49 mA, slice thickness 0.6 mm, pixel size 0.516 mm, Field of View 26.3 cm) were used to investigate the extent of the osteological damage and to provide an accurate image of the state of the internal structures of the bone in order to establish the criteria to develop a differential diagnosis of the possible pathological processes that caused these lesions.

4. Results

The macroscopic inspection of the *Tegmen tympani* revealed abnormal lesions on the endocranial surface consisting of deep and profuse pits on the bone tissue that left the ear channel exposed (Figures 3 and 4). Both lesions presented a polished patina on its surface, as well as on the area surrounding the pathology. No similar lesions were found on the comparative sample of 17 recent temporal bones or on the 12 prehistoric samples. Moreover, the lesion exhibited a focal area of osteoclastic activity (bone destruction), with no sign of osteoblastic activity (bone formation) (Figure 5). In addition, no clear evidence of taphonomic nor pseudopathologic modifications were identified (such as jagged, irregular and sharp edges), the fact that similar lesions appear symmetrically on both temporal bones, and the uniqueness of the modification within the skeletal remains recovered, we determined a pathological origin of the lesions on the La Llana specimen.

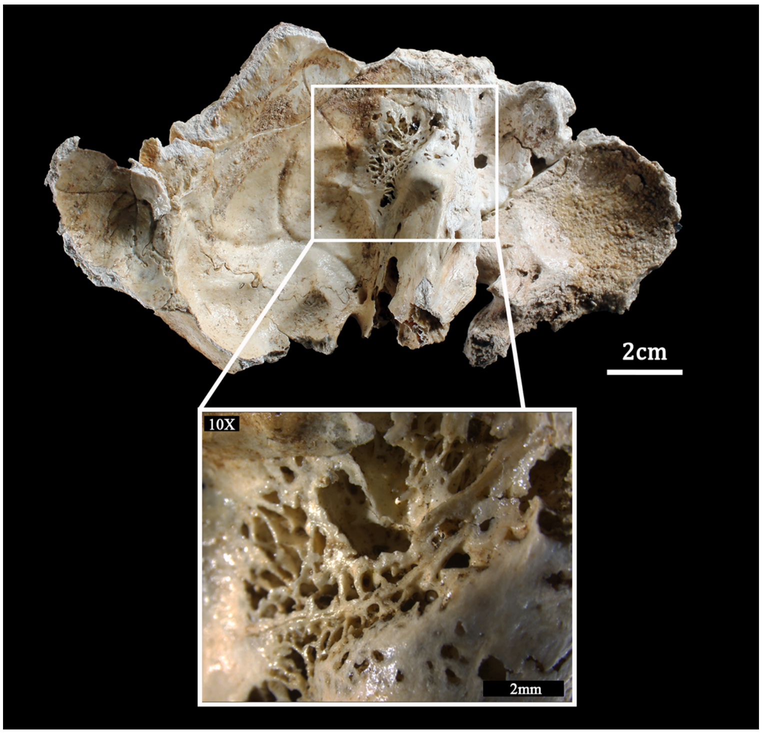

Figure 3: **Osteolytic lesions:** Intracranial view of the individual from La Llana showing the osteolytic lesions on the right *os temporale*.

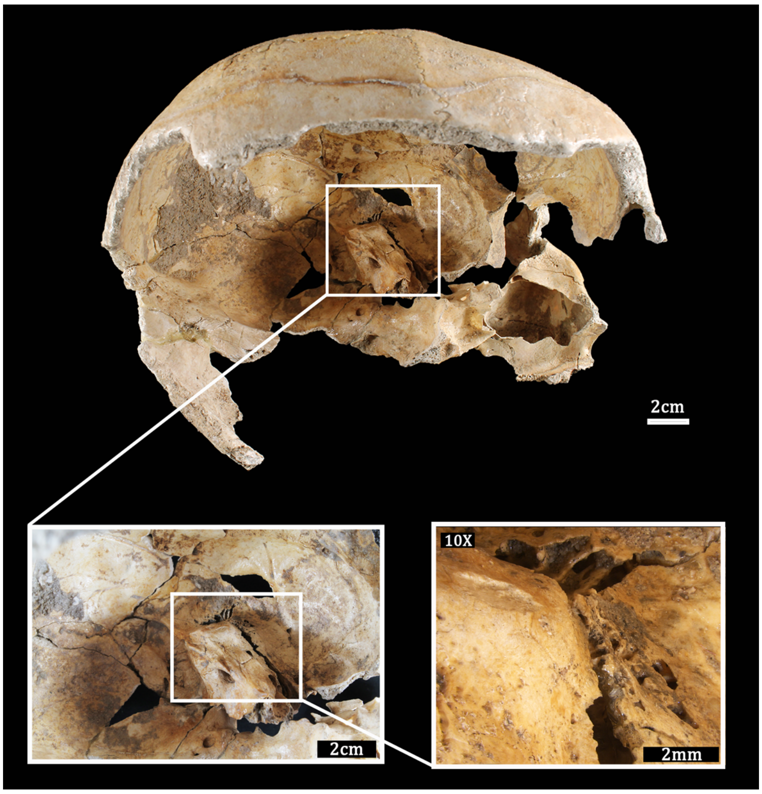

Figure 4: Osteolytic lesions: Intracranial view of the individual from La Llana showing the osteolytic lesions on left *os temporale*.

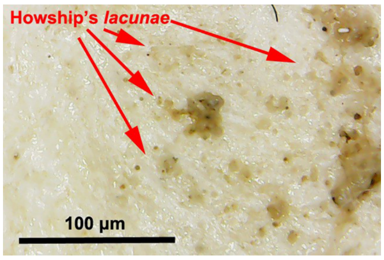

Figure 5: Osteoclastic activity: Surface histology showing Howship's *lacunae* with bone resorption areas.

CT-scans showed that the cortical bone on the *Tegmen tympani* of the right temporal bone had completely disappeared and was perforated, causing the destruction of the attic wall, while on the left temporal bone it was still present (Figures 6A and 6B). Other bone structures of the external and inner ear appeared to be totally intact, such as the *meatus acusticus externus* or the inner canal, which appear to be normal. Pneumatisation cells of the mastoid process show normal morphology (Figure 6C), as well as the cochlea.

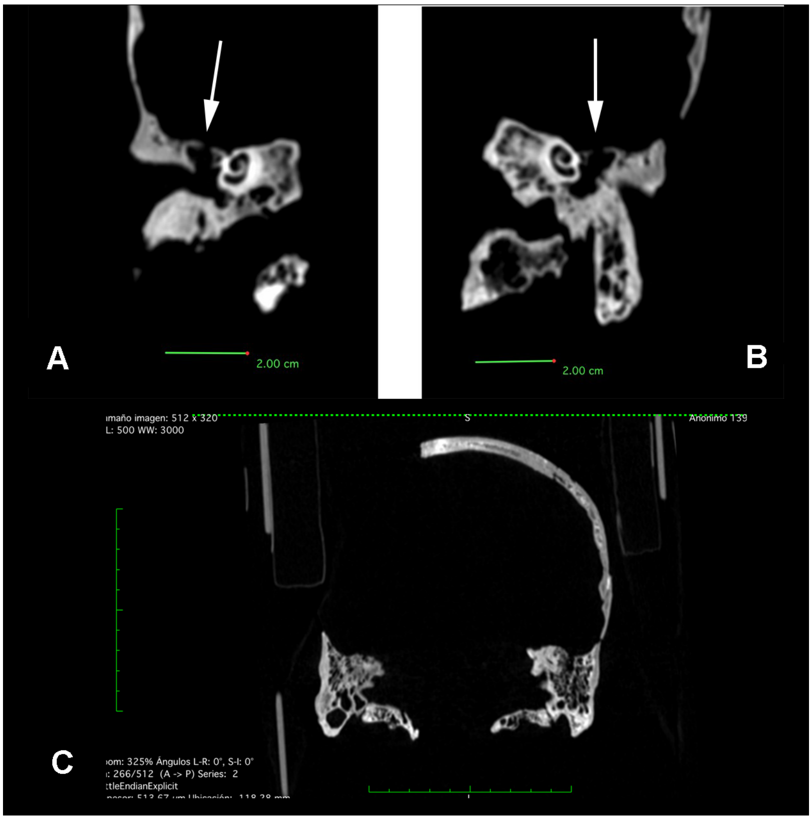

Figure 6: **Computer Tomography:** Coronal CT of the middle ear showing the perforation of the *Tegmen tympani* and the healthy appearance of the cochlea on both the right (**A**) and left (**B**) *os temporale*. The arrows point to the lesion. **C:** Coronal CT of the mastoid process showing normal morphology of the mastoid air cells.

<u>Differential diagnosis</u>

Lesions frequently affecting the temporal bone include mastoiditis (infection of the mastoid air cells surrounding the inner and middle ear), labyrinthitis (inflammation of the labyrinth) and petrositis (inflammation of the apical portion of the petrous temporal bone). However, the regular appearance (following the examples provided by Mann and Hunt, 2012) of the surrounding bone (Figure 6B), would suggest that conditions causing a surrounding osteoblastic or osteoclastic response such as mastoiditis, labyrinthitis and petrositis may not have been involved in the development of the osteolytic lesion detected here. No external auditory exostoses were observed.

Another lesion that can cause the thickening of the *Tegmen tympani* is a meningioma, frequently characterized by the presence of an intratympanic mass that envelopes the ossicular chain and causing the obstruction of the Eustachian tube, and thus spreading the tumor into the middle ear cavity (Hamilton et al., 2006; Nicolay et al., 2014; Ricciardiello et al., 2015, and references therein). In this case, the erosion was not extended to the middle ear cavity, so it is not possible that a meningioma was responsible for both lesions.

Chronic otitis media is a rather common disease frequently linked with a limited pneumatisation of the mastoid process and can produce other subsequent damages on the temporal bone surface such as cholesteatomas (Armentano et al., 2014). A cholesteatoma is a benign tumour widely found within the attic wall that results in increased external ear canal diameters and eroding or sclerosed mastoid processes. Acquired cholesteatoma can cause a retraction of the *pars flaccida*, displacement of the ossicular chain, and invasion of the mastoid process, although sometimes cholesteatoma can reach the *petroux apex*, (Maroldi et al., 2001). The intact appearance of these structures on the La Llana individual indicates that this pathology may not be involved on the bone modification observed.

Despite the lack of cholesteatomas, the osteological modifications observed on both temporal bones are compatible with traces left by a bilateral chronic otitis media infectious process, particularly with atticitis. Bilateral otitis media is a rare disease in adults, but some cases have shown that an individual already suffering from a bacterial or virus infection (i.e. laryngitis, sinusitis) is prone to develop this condition, which can also cause progressive hearing loss (Colodny et al. 1986).

In this case, the infection reached the bone tissue, destroying the cortical bone on the *Tegmen tympani* on both petrous bones, completely in right petrous bone and partially in the left one. In fact, histological observation indicates that the surface of the right petrous bone shows evidence of osteoclastic activity (i.e. bone destruction) and no bone formation was observed, meaning no healing had occurred at the time of death of the La Llana individual. In addition, bone descruction indicates that the infection could have spread into the middle cranial fossa, possibly affecting the meningeal vessels.

5. *Discussion*

The results of the analyses performed on the lesions detected on the Early Broze Age cranium found at La Llana cave points towards a diagnosis of otitis media. Otitis media is an inflammation of the middle ear (Mansour et al., 2013) preceded by an upper respiratory tract infection. Middle ear infections occur as a result of a malfunction of the Eustachian tube, the duct that connects the middle ear with the throat and serves to balance the air pressure between the outer ear and the middle ear. If this duct does not work properly, normal fluid drainage from the middle ear does not occur, and it accumulates behind the eardrum. This favours the development of bacteria and viruses in the ear, which usually cause acute otitis media, an infectious disease very common in children with approximately 80% of them experiencing a case during their lifetime (Lieberthal et al., 2013; Schilder et al., 2016; Danishyar and Ashurst, 2020). Adults can suffer from otitis media-related infections but these are generally episodes of chronic otitis media (McKenzie and Brothwell, 1967; Schilder et al., 2016). A systematic review on the prevalence of otitis media for all ages estimated the rate ranges from an average of 3.6 for central Europe to an average of 43.4 for Sub-Saharan West Africa and central Africa, varying largely due to access to medical care (Monasta, 2012; Schilder et al., 2016). Middle ear inflammation is identified in skeletal remains as erosive lesions and new bone formation of auditory ossicles (Krenz-Niedbała and Łukasik, 2017), as well as more serious subsequent *sequelae* in intratemporal (mastoiditis, labyrinthitis and petrositis processes) or intracranial (meningitis and brain abscess) infections (Casselbrant and Mandel, 2010; Chole and Sudhoff, 2010). Recently, Steyn and Buskes (2016) reported that a case of destruction of the cranial base originated from possible otitis media. The authors also identified alterations of the posterior, middle and anterior cranial *fossae* that are most likely associated with tuberculosis or meningitis (Steyn and Buskes, 2016). In the La Llana individual, these *sequelae* and infectious complications were not detected in CT scans of the *cochlea* and auditory canal. In addition, microscopic examination of the intracranial bone surface identified the presence of Howship's *lacunae* indicating an active process of bone destruction and no healing before death, meaning that the infection was still active at the time of death. The lack of antiobiotic treatments available today meant the infection spread past the *Tegmen tympani* into the meningealvessels. Modern medical interventions has made these complications rare today (0.32/100,000), based on a clinical study of adults from Finland (Leskinen and Jero, 2005). In the case of the La Llana individual it is plausible that spread of the infection into the middle cranial fossa by the destruction of the *Tegmen tympani* and the lack of modern treatment could have developed into intracranial complications such as meningitis or even a brain abscess.

Infection involving the temporal has a long history in human populations, dating at least by to the Middle Pleistocene as demonstrated by the 110kya Kabwe 1 skull from Broken Hill (Bada et al. 1974). This case was diagnosed as mastoiditis and a probable case of granuloma (McKenzie and Brothwell, 1967; Montgomery et al. 1994). Chronic otitis media has also been diagnosed in the Late Pleistocene in an adult Neandertal skull from Qafzeh cave in Israel (Arensburg and Nathan, 1972; which ranges from ~90 to 100 kyr bp according to Valladas et al., 1988 and Schwarcz et al., 1988), as well as in two Gravettian Anatomically Modern Humans from Dolní Věstonice in the Czech Republic (DV14 and DV 15, dated approximately 26kya, Lisoněk and Trinkaus, 2006), both manifested by bony lesions on the surface of the auditory ossicles. Other cases of infectious otopathologies in past human populations have been found in Prehistoric Chilean contexts (Goycoolea et al., 2019), Prehistoric Native Americans (Gregg et al., 1965; Gregg and Steele, 1982; Schultz, 1979; Homøe and Lynnerup, 1991; Mann et al., 1994; Homøe, 1997; Mann and Hunt, 2012; Ochi et al., 2018), early Neolithic (Purchase et al., 2019) and Bronze Age (Daniel et al., 1988) individuals from Europe, Egyptian mummies (Lynn and Benítez, 1974; Horne et al., 1976; Mann, 1992), and skeletons from European Middle Age period (Schultz, 1979; Qvist and Grøntved, 2001; Flohr and Schultz, 2009; Krenz-Niedbała and Łukasik, 2017). The pathologies described in all these populations include mastoiditis, chronic otitis media, labyrinthitis and other inner, middle and external ear infections, indicating that these diseases were not infrequent during the pre-antibiotic era. Despite all the cases reported for prehistoric times, only a few examples have been reported in Spain. The oldest is a left mastoidal fistula from a Neolithic individual from Nerja cave (Malaga) (García Sánchez, 1996).

Bronze Age cases of mastoiditis and cholesteatomas were documented at Cementeri dels Moros, Torrent dolmen (Gerona) and Cova del Pas (Minorca) (Vives and Campillo, 1980; Campillo, 1994; Armentano et al., 2014). Skeletons from the Punic-Roman Necropolis of Cadiz showed different otologic diseases from temporal bones (Macías et al., 2013). The case reported here provides the first example of a middle ear infection for Northern Spain, and one of the first diagnosed using multiple imaging technique to characterize the crucial intratemporal region. However, the presence of this type of lesions in the prehistoric populations of the Cantabrian region must have been more common and probably their absence from the anthropological record is due to a factor of underreporting. Identification of otitis media in ancient human skeletal remains is a difficult task due to the complexity in distinguishing between taphonomical alterations and pathological erosions (Qvist and Grøntved, 2001). In addition, most cases of middle ear disease has been identified in the archaeological record based on indirect evidence rather than clinical-based observation (e.g., Gregg 1982; Homøe and Lynnerup, 1991; Homøe, 1997; Goycoolea et al. 2019, among others). This investigation reintroduces the clinical progression of this condition into the study of otitis media and tests the criteria used for its identification on a separate sample of temporal bones from a similar taphonomic context.

The etiology of otitis media is multifactorial, including several interrelated causes such as host factors (allergy or genetic predisposition), physiological features related to the Eustachian tube, already existing viral and bacterial infections that can spread to the ear (such as sinusitis or laryngitis), and environmental conditions, such as altitude and humidity (Colodny et al. 1986; Casselbrant and Mandel, 2010; Daniel et al., 1988; Ilechukwu et al., 2014;

Qureishi et al., 2014; Zhang et al., 2014). The La Llana archaeological cave site is located on a marine limestone platform 1km from the current coastline, 32m above sea level, thus is exposed to high humidity, which increases the risk of developing otitis (Daniel et al. 1988), and strong coastal winds. During the Early Broze Age, around 3,300 BP, the area was characterized by a marine climate with moderate temperatures, with heavy rains and cold seawater (González Morales,1995). Thus, the La Llana individual was living in an environment conducive to developing this disease. Still, evidence of subsistence strategies from Cantabrian Bronze Age populations discard the possibility of regular exploitation of coastal and marine resources (Arias and Armendáriz, 1998), quite unlike the Mesolithic in the same area (Gutiérrez-Zugasti, 2008), but the anthropological record from the Mesolithic in Northern Spain (mostly from intentional graves) does not show any paleopathological evidence of otitis media (Drak and Garralda, 2009). Aquatic resource procurement has been linked to the development of external auditory exostoses (Villote and Knüsel, 2016; Smith-Guzmán and Cooke, 2019), which in turn is related to otitis externa (Strauss and Dieker, 1987). In addition, no exostoses were found associated with the case reported here.Whilst there is no indirect evidence that this individual engaged in marine activities, it is possible that living in an oceanic environment could be one of the factors affecting the development of the disease in the La Llana individual (Tos and Poulsen, 1979; Cauwenberge, 1984; Ishidoya et al., 1987), and that otitis media has many other causes than the environment (and thus may appear without exostoses) such as genetic predisposition (Padia et al., 2017), as clinical evidence suggests.

Our results indicate that a multifaceted methodological approach, i.e. the integration of macroscopic (*de visu*), microscopic (identification of remodelling surfaces on the lesion) and CT-scans (examination of the internal structures, and adjacent regions) has shown the potential to provide a complete toolkit for study of skeletal lesions. In this research, the most useful method for diagnosis was the CT-scan as it provided information not just on the internal extent of the lesion, but the state of adjacent regions and internal structures, which are otherwise impossible to observe when the bone is not broken.

6. Conclusions

We have examined the skeletal signs compatible with a case of otitis media (atticitis) in the temporal bones of an adult male from the Cantabrian Bronze Age buried in La Llana cave (Asturias, Northern Spain). Macroscopic, microscopic and CT-Scans analyses show evidence of osteoclastic activity and perforation of the *Tegmen tympani* in both petrous bones, and likely intracranial complications such as meningitis or brain abscess. The multifaceted methodology used provided a complete view of the effects of otitis media on bone, making it possible to identify signs of the disease even on fragmented temporal bones. Despite the environmental conditions during the Bronze Age in the Cantabrian region, this disease is a relatively scarce pathology documented in the paleopathological research. This case study constitutes the first reported evidence known for the Cantabrian Region (Northern Spain) until now.

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