

## Case study

## Calcified uterine leiomyoma from an 18th-century nunnery in North Italy

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## ABSTRACT

**Objective:** To develop a differential diagnosis of a mass retrieved alongside skeletal remains in the crypt of the church of Santissima Annunziata of Valenza (Province of Alessandria, Northern Italy).

**Material:** A calcified mass measuring 40 × 39 mm and 17.62 × 16.3817.62 × 16.38 mm.

**Method:** The analysis utilized macroscopic assessment and histologic examination (including histochemical and immunohistochemical analyses).

**Results:** Morphological traits include an irregular and spongy external surface. Holes of different sizes lead toward the inner part of the object. A section of the mass shows an “intertwined bundle” pattern, confirmed by microscopic examination.

**Conclusions:** Differential diagnosis determined the mass to be consistent with calcified leiomyoma.

**Significance:** Identifying uterine leiomyoma adds to the paucity of paleopathological literature on the condition and to calcified tumors more broadly. It also allows for an important discussion of women’s gynecological health in the past and potentially among nulliparous women.

**Limitations:** Neither histochemical staining nor immunohistochemical analysis demonstrated the certain muscular nature of the specimens due to the rehydration and decalcification processes, for which there are no gold standards.

**Suggestions for further research:** Calcified masses are common in the clinical literature but remain rare in paleopathological literature. Careful excavation and improved recognition of apparently calcified masses are necessary to improve recognition, diagnosis, and interpretation.

## 1. Introduction

In 2021, an anthropological study was conducted on the skeletal remains in the crypt of the church of Santissima Annunziata of Valenza in the Province of Alessandria, Northern Italy (Fig. 1) (Fusco and Tesi, 2019). The cemetery was exclusively used by Augustinian nuns from a nearby nunnery and it can be accessed through a trap door on the church floor. The inspections conducted yielded 29 tombs dating to the 18th century. Here, the nuns received a particular type of burial. The deceased were seated in burial cells, supported by a wooden stick at chest level. A vase was placed beneath the seat to collect decomposition fluids, and the cell was then sealed with engraved mortar indicating the burial date (Fusco, 2023). The construction of the crypt coincides with the construction of the church in 1699. Upon entering the crypt, visitors encounter a small access room measuring 3.90 m by 2.50 m, with burial

cells lining the walls. The second room is a long, narrow space approximately 44 m by 3.20 m, featuring cells along its perimeter. This room leads to a third compartment measuring 4.20 m by 2.47 m, which contains additional tombs. All the cells had a step, and the nuns were buried in a seated position on it, supported by the wooden stick, before being walled up.

In 1802, the church of the monastery was closed, and the crypt was filled with soil. However, in 1973, members of the Brotherhood of San Rocco discovered and cleaned the crypt, revealing the tombs (Fusco, 2018). The 2021 bioarchaeological investigations focused on retrieving the complete osteological sample from the tombs to reconstruct the biological and paleopathological history of the nuns of Valenza. In the excavation process, an atypical calcification was identified in Tomb 17 on the supporting surface of the step where the body was positioned. Here, we examine the morphological and histological characteristics of

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the calcified mass in order to develop a differential diagnosis and offer insight into the life and death of the individual in Tomb 17.

## 2. Materials and methods

Inscriptions above Tomb 17 indicate that the nun died in 1779; unfortunately, the individual's initials of their name are illegible (Fig. 2). Fragments of the skull, trunk, and upper limbs were located on the support surface of the step of the tomb, while those of the lower limbs and feet were situated at the bottom of the cell.

Overall, the skeleton was incomplete, in poor condition, highly fragmentary, and brittle due to the humidity of the crypt. Sex was determined based on the morphological characteristics of the os coxae (Phenice, 1969; Acsadi and Nemeskeri, 1970; Bruzek, 2002). The age at death was estimated through degenerative changes of the auricular surface and pubic symphysis (Brooks and Suchey, 1990; Buckberry and Chamberlain, 2002).

The mass under investigation was recovered resting on the skeletal pelvis, measured  $4 \times 3.4 \times 3.9$  cm, and was brown, likely due to decomposition of the body and the surrounding environment. The mass appears solid, but it was very fragile and broke easily into small pieces (Fig. 3).

The entire mass was subjected to two cuts to the central portion to obtain an approximately 0.5 cm-thick section. In order to perform a histologic examination, some fragments were rehydrated. The samples were prepared according to the technique described by Fulcheri and Ventura (2001), with slight modifications. Briefly, the specimens were immersed in Sandison solution (composed of 95% ethanol, 1% formaldehyde, pH 7.4%, and 5% sodium carbonate) for 12 days. After this, they were decalcified in a formic acid decalcifier for 24 h, then dehydrated (24 h in 80% alcohol, 24 h in 95% alcohol, and 24 h in 100% alcohol), diaphonized (24 h in xylene's substitute), embedded in paraffin, sectioned, and stained with hematoxylin and eosin, and Masson's trichrome for histological analysis (including immunohistochemistry with pancytokeratin and desmin). Another fragment was embedded in epoxy resin and stained with hematoxylin and eosin.



Fig. 2. Walled tombs around the edges of the second crypt space, with Tomb 17 positioned in the center. Observe the inscriptions at the top of the cells.

## 3. Results

### 3.1. Morphological observation

The skeleton from Tomb 17 belonged to an adult female over the age of 50. On the surface of the bones, powdery deposits of whitish material are visible, which could be deposits of calcium salts from the burial environment.

The external surface of the mass under investigation is irregular and suggests an “intertwined bundle” organization; moreover, it has a spongy appearance, created by multiple irregular spaces separated by



Fig. 1. Map indicating the site's location within Italy.

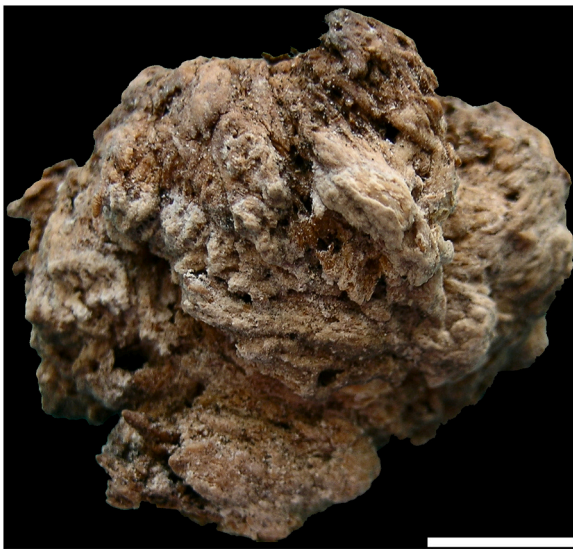


Fig. 3. Unidentified concretion retrieved from Tomb 17.

small trabeculae. Holes of different sizes, ranging from 1 mm to 3 mm, can be observed that lead toward the inner part. The cut surface of the mass confirms the “intertwined bundle” pattern (Fig. 4).

### 3.2. Histological results

Upon microscopic examination, there is further confirmation of the intertwined pattern, often associated with intersecting fascicles of spindle cells with indistinct borders, probably associated with hyalinization and rare microcalcification (as observed in longstanding neoplasms) (Fig. 5). Neither Masson’s histochemical staining (uniformly reddish, without blue-colored fibers) nor immunohistochemical analysis with anti-pancitokeratin and anti-desmin antibodies (both negative) demonstrated the muscular nature of the specimens (possibly due to the rehydration and decalcification processes, for which there are no gold standards, and due to the poor state of preservation of the sample).

## 4. Discussion

Macroscopically, the mass presented as a concretion, and subsequent histological examination revealed its organic origin. A calcific concretion, entirely mineral, cannot be processed using histological/paleohistological techniques. This, coupled with the observed morphology,



Fig. 4. Cross-sectional view of the concretion following histological sectioning.

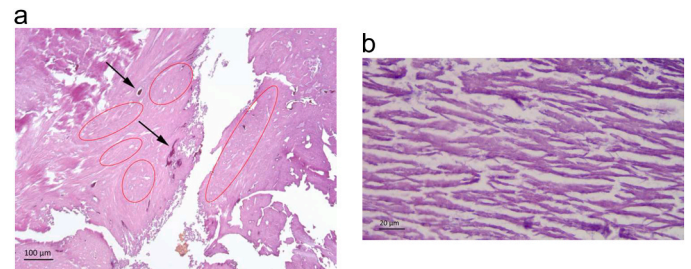


Fig. 5. a) Microscopic examination of decalcified sections of the concretion in paraffin after rehydration with Sandison solution. Intertwined patterns indicating intersecting fascicles of spindle cells with indistinct borders are denoted by red circles. Rare microcalcifications are marked with black arrows. b) decalcified sections embedded in epoxy resin. Morphological details are somewhat limited likely due to poor preservation.

led to the dismissal of the possibility of the mass being mineral in origin and, instead, suggested an organic origin. The histological analysis presented a growth pattern of intertwined bundles, “whorls-like”, which corresponds to the appearance of calcified masses of a neoplasm of muscular origin.

It is important to note that Masson’s trichrome staining did not reveal bluish fibers (associated with collagen or muscle tissue), and that immunohistochemical analysis (negative for both cytokeratins and desmin) does not determine with certainty a muscular origin of the tissue. This may be due to multiple factors, including poor preservation of the material and further alterations due to rehydration and decalcification processes.

The data obtained from the analysis reveals that it is consistent with a calcified mass with no evidence of osseous change. No bone tissue structure was observed in the thin section (Kramar et al., 1983; Lagier and Baud, 1980). Given the seated position of the corpse, determining the precise origin of the calcification proves challenging. The calcification could have originated in the pelvic or chest region, later descending into the abdomen during the body’s decay. Hence, calcifications from various body parts were considered in the differential diagnosis. Fortunately, some possibilities were immediately excluded based on external morphology, object size, and the individual’s sex. Initially, we considered thoracic tumors consisting of fibrohyalin connective tissue, such as solitary fibrous tumors and pulmonary hamartomas. Following that, we considered lymph nodes, hydatid cysts, osteosarcomas, calcified ovaries, bezoars, bladder stones, and uterine leiomyomata.

### 4.1. Solitary fibrous tumor

The calcification of a solitary fibrous tumor has been documented at various anatomical sites, including the pleura, mediastinum, heart, lung, neck, mandible, spine, back, arm, and thigh, as well as oral, inguinal, para testicular, and intrascrotal locations. These tumors contain hyalinized collagenous stroma, but histologically are composed of haphazardly organized fascicles, less defined and shorter than our calcification (Tariq et al., 2021).

### 4.2. Pulmonary hamartoma

Pulmonary hamartoma is a benign neoplasm composed of cartilage, connective tissue, muscle, fat, and bone. It typically presents as well-circumscribed nodules or masses with either smooth or lobulated margins. Approximately 60% of these neoplasms contain fat and about 20–30% exhibit calcification, resembling popcorn kernels. Their size varies from larger than 10 cm, but most measure 2.5–4 cm in diameter. Our calcification did not display lobulated margins and popcorn-like ossification (Siegelman et al., 1986).

#### 4.3. Lymph node

Calcification of a lymph node may arise due to infections like tuberculosis, sarcoidosis, or neoplasms such as lymphoma or carcinoma (Gawne-Cain and Hansell, 1996). Lymph nodes may exhibit calcification resembling an eggshell, typically accompanied by vessel impressions in these swellings (Komar and Buikstra, 2003). However, the calcification under investigation does not exhibit these features.

#### 4.4. Hydatid cysts

Hydatid cysts are caused by *Echinococcus granulosus*. Typically, these cysts are multilocular, water-filled, and can reach a diameter of 15–20 cm. However, we can exclude them from our considerations because they calcify, forming an eggshell-like structure with a hollow center that remains uncalcified (Aufderheide and Rodríguez-Martín, 2014; Monge Calleja et al., 2017).

#### 4.5. Osteosarcoma

Osteosarcoma can occur in the ovary or uterus and, although rare with only a few reported cases, is the most common primary malignancy among bone tumors (Yeasmin et al., 2009). The size of the tumor can vary from 5 to 27 cm. Histologically, osteosarcoma exhibits visible osteons with bone formation and focal calcification, which can be detected through radiographic imaging (Hardisson et al., 2001; Hirakawa et al., 1988). Lacking these features, we can rule out osteosarcoma as a potential diagnosis.

#### 4.6. Calcified ovaries

Torsion or a local vascular event normally leads to autoamputation followed by the ovary becoming migratory and presenting as a calcified mass (Fletcher et al., 1988; Kennedy et al., 1981). Reported sizes of surgically removed calcified ovaries have ranged from 10 × 22 × 30 mm (Lester and McAlister, 1970) to 18 × 23 × 30 mm (Kennedy et al., 1981). Others have measured 25 × 20 mm and 35 × 25 mm (Fletcher et al., 1988). The nodule recovered from Tomb 17 does not fall within this range. Although calcified ovaries are more frequently reported in children in modern literature, they are considered a rare pathology (Lester and McAlister, 1970; Kennedy et al., 1981; Fletcher et al., 1988).

#### 4.7. Bezoars

Bezoars (stomach stones) are accumulations of indigestible contents within the gastrointestinal tract. The formation of stomach stones may also be a defense mechanism against damage caused by the ingestion of a foreign body. A bezoar can be made up of indigestible material from fruits and vegetables, such as fibers, peels, seeds, hairs, and food particles. The absence of these materials and the intertwined bundle structure of the calcification under investigation allows for its exclusion (Rothschild and Biehler-Gomez, 2021; Tomassini et al., 2019).

#### 4.8. Bladder stones

Bladder stones typically have an ovoid or spherical shape, varying in size from a few millimeters to several centimeters. Larger stones are categorized as giants (Gładkowska-Rzeczycka and Nowakowski, 2014). They exhibit a consistently colored surface, ranging from white to dark brown, with a generally smooth texture occasionally featuring small papillations. Internally, the structure is laminated, characterized by crystalline layers with slightly different compositions converging on a central core (Dabbs, 2023). The calcification found in Tomb 17 can be distinguished from bladder stones, as it lacks the typical concentric layers seen in vesical calculi (Anderson, 2003).

#### 4.9. Uterine leiomyomata

After considering potential etiologies, a diagnosis of calcified uterine leiomyomata can be suggested. The size, spherical shape, irregular surface, and coarse trabecular texture are characteristic of this condition (Kramar et al., 1983; Strouhal and Jungwirth, 1980). Furthermore, the fact that the calcification was found in a female skeleton supports the diagnosis of uterine fibroids. Uterine leiomyoma, also known as a fibroid, is a common benign neoplasm that develops within the smooth muscle tissue of the uterus and has been associated with hormonal activity, particularly estrogen. Uterine leiomyoma tends to increase in size during periods of high estrogen levels, such as reproductive years, and decrease in the presence of low estrogen levels. These types of neoplasms affect 30–50% of women of reproductive age (Kumar et al., 2017). Myomas are rarely seen in women under the age of 18 and tend to regress after menopause (Kramar et al., 1983; Lagier and Baud, 1980). Sizes range from small to large (Coronado et al., 2000; Katz et al., 1989; Mettler et al., 2012; Scheidegger, 1990). The number of myomas can also vary from one to three in the same individual, although cases with more than 50 tumors in the uterus have been reported (Bégouin and Papin, 1937).

While uterine leiomyomas are benign, they can have adverse effects on the individual such as hemorrhagic disturbances, leading to anemia, potentially impacting fertility, or causing miscarriages (Coronado et al., 2000; Katz et al., 1989; Mettler et al., 2012). They can also lead to secondary anatomical changes, particularly when the vascular supply is disrupted, leading to degenerative alterations (Lopes et al., 1982; Jiménez-Brobeil et al., 1992). The specific type of degenerative change appears to depend on the degree and speed of vascular insufficiency onset (Haines and Taylor, 1962).

Various forms of degeneration have been identified in uterine fibroids, with hyalinization being the most common (Ho et al., 2007). A comprehensive study revealed that 26.5% of all myomas exhibited hyalinization (Miller et al., 2020). Hyaline degeneration can sometimes progress to cystic degeneration, leading to the formation of a more intricate cystic mass within the fibroid (Miller et al., 2020; Wilde and Scott-Barrett, 2009). In about 4% of hyalinized fibroids, calcification can occur. Typically, the calcifications are dense and amorphous, resembling dystrophic calcifications seen in other parts of the body. However, there are instances where rim calcifications can develop, as well (Ho et al., 2007).

The case from Tomb 17 is uncommon considering that only approximately 4% of myomas undergo calcification, and the prevalence of myomas in women over the age of 35 is around 20–25% (Cole et al., 2015). Some authors propose that uterine myomas are typical in nulliparous women, but this statement is not universally accepted within the medical community (Jiménez-Brobeil et al., 1992). It is interesting to note that the case under analysis was found adjacent to the skeleton of a nun; a plausible hypothesis would be that this woman never experienced pregnancy. Similar cases described in the literature, such as the studies conducted by Quintelier (2009), where three fibroids were found in the same convent, often involve nuns or women who never bore children. The presence of a calcified uterine leiomyoma next to a nun's skeleton, as in our case, could thus serve as additional confirmation of this trend observed in other archeological discoveries. It is important to emphasize that even though this study presents a single instance, it supports the assertion that nuns or nulliparous women might have a higher predisposition to develop calcified uterine leiomyomas. However, further research is essential to delve deeper into this correlation. Despite the low prevalence of calcified uterine leiomyomas and the challenges associated with their discovery in archeological findings, they have been noted in the paleopathological literature (Table 1). It is plausible that, like contemporary women, those in the past may have experienced the development of uterine leiomyomas with comparable side effects. Unfortunately, the lack of information regarding the medical history of the woman in Tomb 17 makes it impossible to determine

**Table 1**  
Leiomyomas reported in the paleopathological literature\*.

Maximum dimensions (mm)	Age of individual	Period	Location	References
123 × 105 × 80	35–45	3rd–4th century CE	Egyptian, Nubia	(Strouhal and Jungwirth, 1980)
56 × 52 × 45		4700–3490 BC	Corseaux-sur-Vevey, Switzerland	Kramar et al., 1983
30 × 26 × 18		5th–7th century CE	Rances, France	(Baud and Kramar, 1990)
70 × 70 × 70		Medieval	Basel, Switzerland	Scheidegger, 1990
46 × 32 × 25		5th–10th century CE	Sion Sous-les-Scex, France	(Baud and Kramar, 1991)
61 × 48 × 48	mature	13th–15th century CE	Grenada, Spain	Jiménez-Brobeil et al., 1992
13 × 13 × 13	~40	6th–4th century	Alfedena, Italy	Capasso et al., 1994
71 × 63 × 47		79 CE	Ercolania, Italy	(Capasso, 2001)
	adult	6th–4th century BCE	Fondillo, Italy	La Verghetta and Capasso, 2001
	~32	2nd–3rd century	Fondillo, Italy	La Verghetta and Capasso, 2001
71 × 47 × 47	40–50	16th–18th century CE	Brussels, Belgium	Quintelier, 2009
53 × 47 × 47	35–40	18th century CE	Brussels, Belgium	Quintelier, 2009
31 25 25	50–60	16th–18th century CE	Brussels, Belgium	Quintelier, 2009
243 × 190 × 164	Mature	Post-medieval	Chichester, England	Cole et al., 2015
25.55 × 18.23	25–35	13th and 17th CE	Mugeni, Romania	Miller et al., 2020
17.62 × 16.38	25–35	13th and 17th CE	Mugeni, Romania	Miller et al., 2020
40 × 34 × 39	50–60	1799	Valenza, Italy	This paper

\* adapted from Cole et al. (2015).

whether she was aware of the tumor's presence during her lifetime. Calcified uterine leiomyomas, such as the one discovered in this case, can manifest various symptoms. While some individuals may remain asymptomatic, others might endure issues such as abnormal uterine bleeding, pelvic pressure, pelvic pain, and reproductive problems (Stewart, 2001). Understanding the complications faced by women in earlier eras is challenging due to limited literacy among women and societal norms that discouraged discussions on sensitive topics.

## 5. Conclusion

Microscopic and macroscopic analysis of the calcified nodule found in Tomb 17 suggests the presence of a calcified soft tissue tumor. The size and micro-morphological characteristics are consistent with a calcified leiomyoma. This finding aligns with the medical literature, indicating that soft tissue calcifications are common in contemporary populations (De Faria et al., 2020; Kwee and Kwee, 2019). Furthermore, paleopathological literature supports the notion that such calcifications also occurred in the past (Binder et al., 2016; Binder and Roberts, 2014). These calcifications play a significant role in disease manifestation and should be considered when studying ancient populations.

Several factors contribute to the limited number of reported paleopathological cases thus far. Often, they are not recognized, and if they are, they are not published or reported because of the difficulty in diagnosing them (Dabbs, 2023). Furthermore, the lower average age at death in the past may have contributed to a lower frequency of calcified fibroids. Moreover, compared to bone, calcified fibroids are more

susceptible to destruction by taphonomic processes, potentially leading to their oversight during excavations. Uterine leiomyoma may be frequently misdiagnosed, but a regular review of paleopathological data, improvement in diagnostic methods, and thorough differential diagnosis in paleopathology can improve our understanding of a uterine leiomyoma's natural history in the past.

Furthermore, the presence of a calcified uterine leiomyoma next to a nun's skeleton, as in our case, aligns with findings from other discoveries associated with nuns or nulliparous women. Interpreting this data is complex and warrants further investigation, especially considering the limited data at our disposal. However, these discoveries not only enrich our understanding of the gynecological conditions of women in the past but also provide crucial historical and social context.

## CRedit authorship contribution statement

**Fusco Roberta:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tesi Chiara:** Writing – review & editing, Validation, Methodology. **Fulcheri Ezio:** Validation, Formal analysis. **Spina Paolo:** Validation, Methodology, Investigation, Formal analysis. **Licata Marta:** Validation, Supervision.

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