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Case study
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Diagnostic Re-Evaluation of "Pleural Sarcoma" in Two Wet Specimens from the Pathology Collection in the University of Turin

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A B S T R A C T

Pathology Museums often house fluid-preserved historical specimens. In recent years, the possibility to study such specimens through modern techniques has increased their importance, due to their biological and historical value. The Pathology Collection of Turin houses around 300 wet specimens dating back to the late 19th and early 20th century. The majority of them are still in their original jars, with labels describing year and diagnosis. Two cases of this collection are labeled as "pleural sarcoma", and date back to years 1896 and 1898. The original post mortem reports are filed in the library of the Pathology Institute of Turin. At present times, primary sarcoma would represent a generical diagnosis and an extremely rare entity in the pleura, where the most common primary neoplasm is malignant mesothelioma. Thus, a diagnostic re-evaluation was carried out, including routine histopathology, and immunohistochemistry, as well as an archive survey of the original reports. The morphological analysis and the available clinical correlates allowed to reclassify such cases as metastatic squamous cell carcinoma from an unknown primary, and pleuro-pulmonary metastases of a uterine leiomyosarcoma. This study suggests that, at the end of 19th century, the post mortem diagnosis of pleural sarcoma encompassed different pathological conditions, often including secondary lesions.

Key words: *pathology museums, wet specimen, lung, sarcoma, histopathology, forensic anthropology*

Introduction

Historical pathology museums often house old anatomical preparations stored in liquid. These precious specimens may show diseases unavailable at the present time, due to modern diagnosis and therapies[1,2]. The opportunity to investigate museum wet specimens with modern techniques has recently increased their scientific importance[3,4].

The pathology collection at the University of Turin houses around 300 wet specimens dating back to the end of 19th century and the beginning of the 20th century[5,6]. Most of the specimens are in their original jars with labels describing year, autopsy number, and diagnosis. In this study, we describe two cases labeled as "pleural sarcoma", a very unusual diagnosis and worthy of re-evaluation. The cases were investigated with modern techniques by minimal sampling, preparation of histological sections, and set-up of histological and immunohistochemical stains.

Material and Methods

Case 1 was labeled as "Sarcoma multiplo della pleura" (multiple pleural sarcoma) and dated back to 1896. No autopsy number was on the label (Figure 1A). Case 2 was labeled as "Sarcomi multipli della pleura" (multiple pleural sarcomas), and the autopsy number was 5226, dating back to 1898 (Figure 1B). The original autopsy records were analysed in order to find out the patients' history and the main gross pathology findings.

The jars were opened and minimal sampling was performed without further damage to the specimens. Tiny biopsies obtained from hidden portions of both lungs were

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Fig. 1. A) Case 1 in its original jar. B) Case 2 in its original jar.

routinely processed for paraffin embedding, serial sectioning, and hematoxylin and eosin (H&E) staining. Additional slides were stained with Masson's trichome, Gomori's method for reticulin fibers, and Papanicolaou method. Immunohistochemistry was performed as well, using a DAKO Omnis™ automated platform. Monoclonal antibodies against smooth muscle-actin (clone HHF35), desmin (clone D33), cytokeratin 7 (clone OV-TL 12/30), cytokeratin AE1/AE3 (clone IR053), vimentin (clone V9), calretinin (clone DAK-Calret 1), cytokeratin 20 (clone Ks20.8), CD68 (clone KP-1), and p40 (clone DAK-p40) protein were employed. Positive and negative controls were performed in parallel.

Results

The specimens had excellent macroscopic preservation. Unfortunately, the external shape of the organs was distorted because of their long persistence within the narrow space at the bottom of the glass jars, and by the presence of superficial cuts most likely performed during autopsy.

Grossly, case 1 specimen contained a left lung attached to the pericardium, with multiple confluent grey-brownish nodules protruding from the visceral pleura (Figure 2A). Case 2 specimen was a left lung containing many round and smooth grey nodules on the surface of the visceral pleura, partially penetrating the lung (Figure 2B).

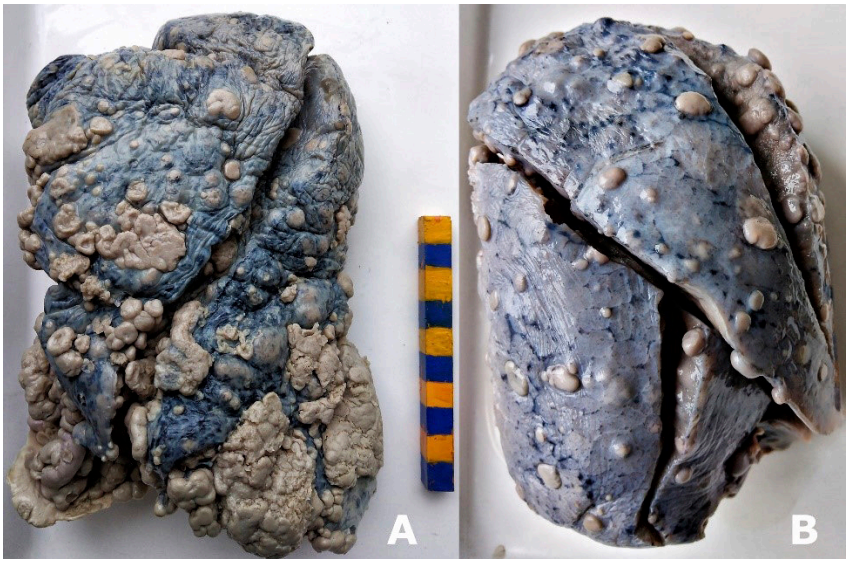

Fig. 2. A) The anterior surface of the left lung, attached to the pericardium, with grey brownish nodular lesions on the visceral pleura. B) The anterior surface of the left lung with round and smooth grey nodules on the pleura.

The label on the jar of the first case reports the year 1896, but not the autopsy number. A survey of the 1896 register identified only two cases of pleural neoplasm. The best fitting patient was a 57 years old female (A. R.) who died on November 27th, the autopsy was performed the day after. The report (no. 4681) described a disseminated neoplasm with pleural involvement (Figure 3).

Microscopically, the neoplasm consisted of both spindle and cuboidal cells arranged in a solid nodular pattern (Figure 4A). Tumor grading showed a range from well to moderately differentiated areas. No foci of keratinization were evident by Papanicolaou stain, but intercellular bridges were focally recognizable in well differentiated areas. Hypercellularity, nuclear atypia, and necrosis were suggestive for a malignant neoplasm. Trichrome staining confirmed intratumoral fibrotic bands (Figure 4B), whereas reticulin fibers circumscribed large groups of cohesive cells (Figure 4C). Alcian blue staining was negative throughout the neoplasm, as well as cytokeratin AE1/AE3, vimentin, calretinin, cytokeratin 20, CD68 and p40. Cytokeratin 7, cytokeratin AE1/AE3, and CD68 were also negative in the neoplastic cells, but focally positive in their respective internal controls (Figure 4D). Therefore, a revised diagnosis of "pleuro-pulmonary metastases of squamous carcinoma of unknown primary" was rendered.

The label on the jar of the second case showed the number 5226, corresponding to the autopsy report of a 94 years old woman (O. L.) who died on January 13th, 1898. The autopsy was performed after six days. The report mentioned a "nodular pleura" and also described a "soft tumor of the uterine cervix metastatic to the liver" (Figure 5).

Histologically, nodular lesions made of pleomorphic and spindle cells arranged in a storiform pattern were found at the pleural surface and infiltrating the lung (Figure 6A). The neoplastic cells appeared pleomorphic, despite a diffusely weak nuclear staining due to untimely fixation and/or acidic fixation/storage fluid (Figure 6B). Focal areas of necrosis were also observed, but no mitotic figures were found throughout the tumor. Scant intra-tumoral fibrous tissue was highlighted by trichrome staining (Figure 6C), while reticulin fibers surrounded single cells and small cellular groups (Figure 6D). Among the neoplastic nodules, compressed, residual, normal alveolar structures were positive for cytokeratin AE1/AE3 (internal control). On the other hand, vimentin, actin, desmin, and cytokeratin AE1/AE3 were negative in neoplastic cells. The revised histological diagnosis was "pleuro-pulmonary metastases of spindle cell and pleomorphic sarcoma, of possible uterine origin".

Discussion

In this study, we reported the histopathological re-evaluation of two cases of claimed "pleural sarcomas" belonging to the ancient pathological collection of the University of Turin, dated from the end of the 19th century. Both specimens were stored in glass jars containing a preservation fluid of unknown composition and were collected during post mortem examinations performed in 1896 and 1898. In the original autopsy records, there was no evidence of a histological examination and it can be assumed that the original diagnosis was based on macroscopic fea-

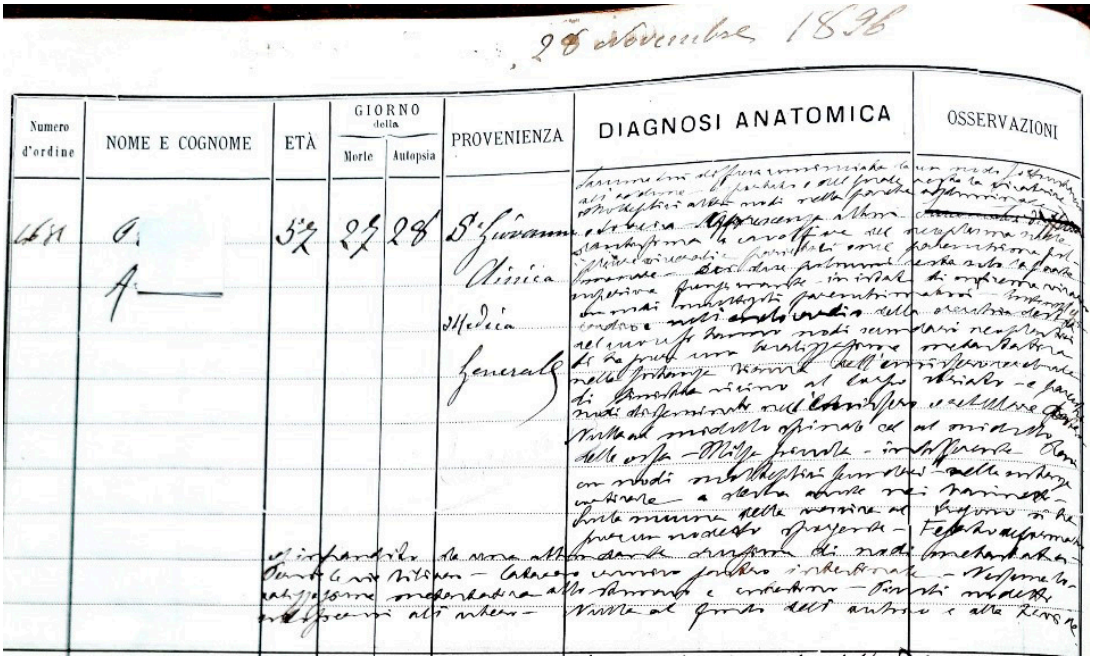

Fig. 3. The original autopsy report of case 1.

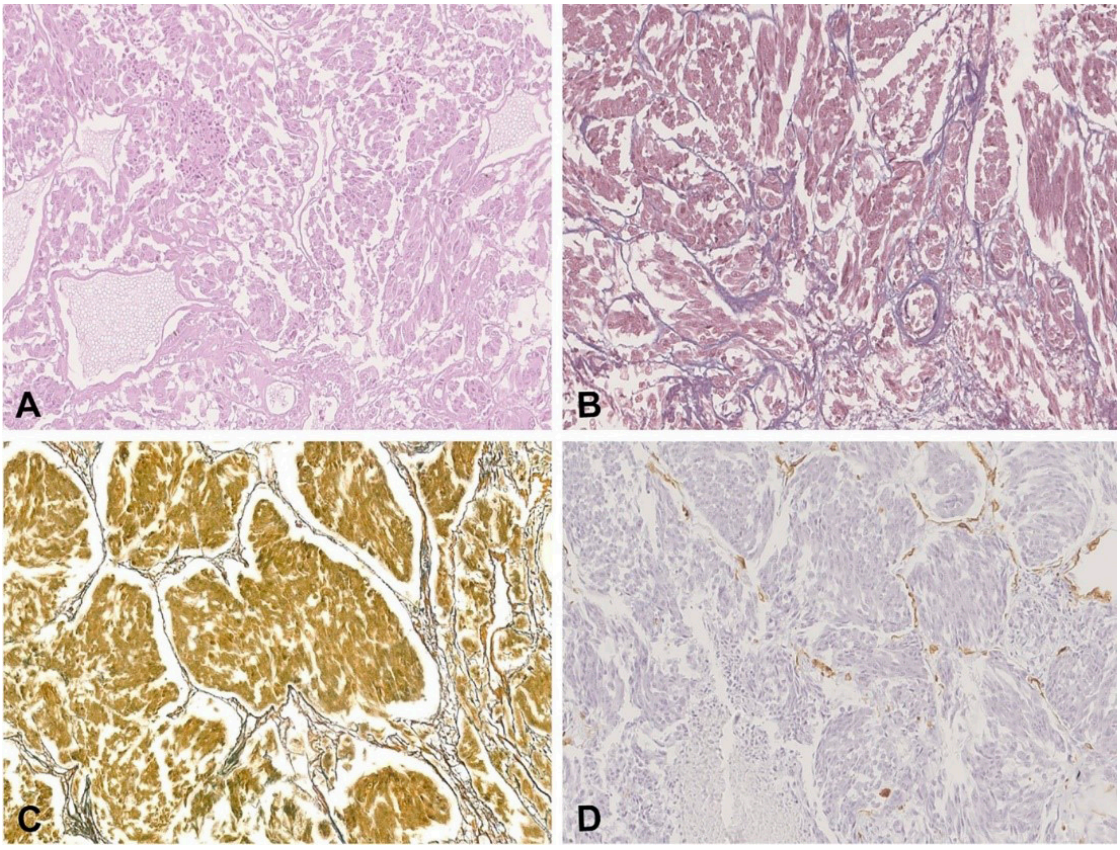

Fig. 4. A) Neoplastic epithelioid polygonal cells arranged in solid nests. (hematoxylin-eosin, original magnification: 100x). B) Intratumoral fibrous tissue (Masson's trichrome, original magnification: 200x). C) Reticulin fibers surrounding cohesive cells (Gomori's stain, original magnification: 200x). D) Neoplastic cells do not express cytokeratin 7 with residual pneumocytes as a positive internal control (original magnification: 200x).

tures, only. Small biopsies were performed on the tumor nodules, care being taken not to damage the whole specimen and a histopathological examination was done, according to the modern diagnostic procedures. Such re-evaluation and the correlation with the corresponding autopsy reports, allowed to re-classify both cases as pleuropulmonary metastases. In the first case, secondary localizations were from a squamous carcinoma of unknown origin and in the second from a spindle cell sarcoma of possible uterine origin. The reason of the original diagno-

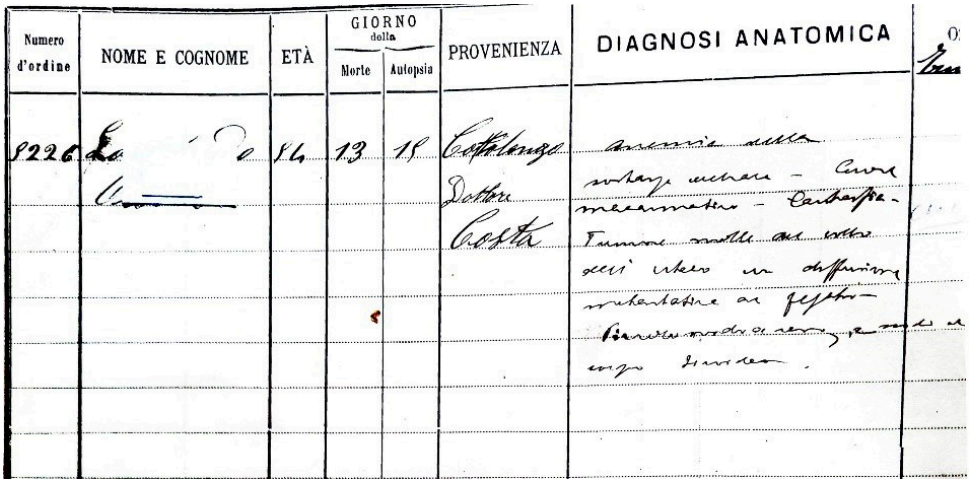

Fig. 5. The original autopsy report of case 2.

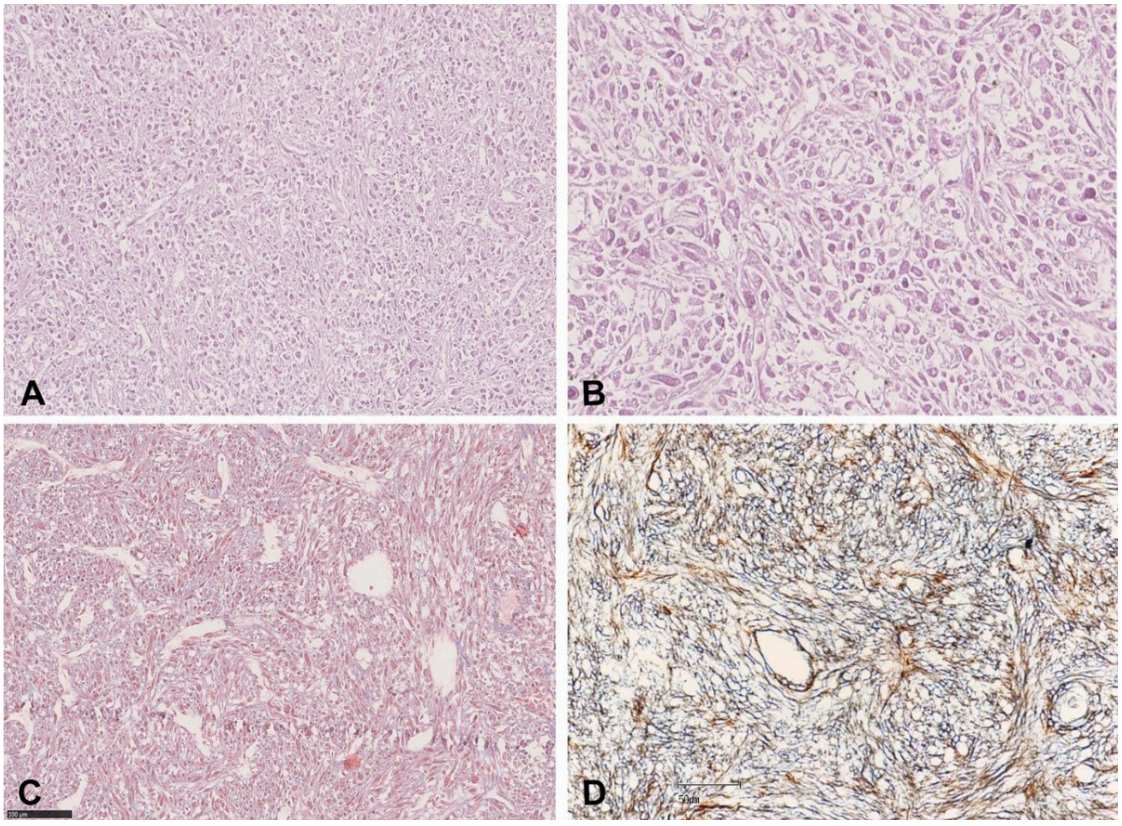

Fig. 6. A) Neoplastic spindle cells arranged in a storiform pattern (hematoxylin-eosin, original magnification: 100x). B) Detail of the tumor showing artifacts due to untimely fixation (hematoxylin-eosin, original magnification: 400x). C) Scant intratumoral fibrous tissue (Masson's trichrome, original magnification: 200x). D) Reticulin fibers surrounding single cells and small cellular groups (Gomori's stain, original magnification: 200x).

sis of "sarcoma" is not clear and is possibly related to the presence of multiple nodular lesions that, without histological support, favored the suspicion of a multifocal mesenchymal neoplasm rather than a metastatic carcinoma. In addition, despite the autopsy report of case 2 clearly mentioned a "soft tumor" of the uterine cervix with liver metastases, the possibility of associated pleuro-pulmonary metastases was surprisingly not considered. This may be due to the lack of knowledge that uterine tumors can also undergo pleuro-pulmonary dissemination. Finally, we cannot exclude that the term "sarcoma" was used at that time as a wastebasket generic diagnosis for otherwise unclassified malignant tumors.

Indeed, true primary pleural sarcomas represent extremely rare lesions. In his textbook of Pathology published in 1921[7], Pio Foà (1848–1923), Professor of Pathology at the University of Turin, and the physician who actually performed the postmortem examination of the two currently reported cases, split "pleural neoplasms" into benign and malignant, with the latter including sarcomas, epitheliomas, and secondary tumors. Two main forms of sarcoma were reported to affect the pleura (generally males younger than 50): round cell and spindle cell sarcoma, including intermediate variants, with no reported macroscopic differences between such forms. Secondary tumors (pleural metastases) were much more frequent than primary neoplasms, and usually included carcinomas (from breast, stomach, lung, esophagus, thymus), but also sarcomas and lymphosarcomas. Mesothelioma is nowadays a well-known primary pleural malignancy, but at the end of 19th century it could not be recognized since the first reports of this tumor date back to 1935[8,9], and this rare entity was probably misplaced by Foà into the categories of epithelioma/endothelioma.

Regarding histopathological examination, both conventional stains and histochemistry were helpful to re-assess the tumor features thanks to a relatively well-preserved morphology. Conversely, immunohistochemistry could not fully support the diagnostic workup, due to the difficulty of antigen retrieval from old museum specimens fixed and stored in unknown fluids. It is well known that formalin fixation may hinder the immunoreactivity of some antibodies, but no firm conclusions can be drawn for tissues fixed and stored for so long in fluids different from formalin and for a long time[10]. In addition, as occurring in modern times especially in autopsy specimens, other factors may affect antigen preservation, including autolytic tissue changes, delayed fixation and type of fixative[11]. Regarding the currently described cases, the original autopsy records revealed that case 1 underwent autopsy the day

after death, but case 2 was examined only six days after death, thus questioning the reliability of antigen preservation, even if cold chambers or refrigerators were in use for temporary preservation of the corpses[12]. Time delay of the autopsies may also well explain the total absence of mitotic figures in tumor tissues and the poor uptake of hematoxylin staining in the histological section. While the delay in fixation may heavily affect cyto-histological details and immunoreactivity, tissue architecture remains generally well-preserved, as clearly demonstrated by histological investigations of different mummified organs in the paleopathology literature[13–15].

The re-evaluation of ancient diseases according to modern classification schemes represents an extremely difficult challenge. Liquid-stored human organs sampled during past autopsies and stored in Pathology Museums are of great paleopathological interest, and represent an invaluable heritage from both historical and cultural viewpoints. However, the study of wet specimens with modern techniques does not always provide reliable results, because the liquids in which they were originally fixed and subsequently stored have a negative impact on antigen and DNA preservation[16,17]. Unfortunately, the composition of fixation and storage fluids is almost always unknown, especially for specimens dating back to 19th century, when formalin was still not routinely used as a fixative[18]. This is the case of our two specimens, as the color of both organs was partially preserved and the characteristic pickle-like pungent odor was missing, thus confirming that the original storage fluid was not formalin.

In the early times of histology, the original fluids employed to fix human specimens were single acids, mixtures of acids, or mixtures of acids and salts[19]. In 19th century, the fluids employed for fixation and storage could be more than a hundred and none included formalin[20,21]. Formaldehyde was discovered in 1859 by the Russian chemist Alexander M. Butlerov, but its use as a biological reagent occurred rather later due to the late development of its manufacture by the chemical industry[22]. A 1/10 dilution of the formaldehyde concentrated (37%–40%) aqueous solution started to be manufactured in Germany in 1891 under the trade names of Formalin or Formol[21]. In 1893, Isaak Blum (1833–1903) noted that formaldehyde would preserve biological specimens[23]. While testing the bactericidal properties of the commercial product formalin (10% formaldehyde), his son Ferdinand Blum (1865–1959) discovered an additional feature of formaldehyde by accidentally fixing the skin of his own fingers. He also found out that this solution was able to preserve and harden samples of liver, kidney, brain and stomach[24]. In Italy, according to the report of Carlo Ascoli in 1894, formalin was only used as an antiseptic. In details, using slips of bibulous paper impregnated with cultures of various micro-organisms, 5% formalin was able to kill Vibrio cholerae, Bacillus anthracis, anthrax spores, Corynebacterium diphtheriae, and Staphylococcus aureus at different times[25].

This historical background indicates that for sure formalin was not the fixative used at the time of the currently reported specimens. Cases from the Turin Collection date back to 1895 and were probably stored in potassium dichromate. According to some original autopsy reports, specimens dated around year 1930 were fixed in Kaiserling solution[26], but no data are available for specimen fixatives used before that time. Since the chemical composition of fixation and storage liquids is mostly unknown, the results of ancillary techniques are largely unpredictable. Thus, histological, histochemical, and immunohistochemical findings call for the integration with the gross pathological data of the original autopsy records, in order to reach a correct diagnostic re-evaluation.

Conclusions

At the end of 19th century, "pleural sarcomas" were probably over-diagnosed due to their macroscopic similarity with secondary lesions. The diagnostic re-evaluation of original reports in museum specimens may be highly informative both for the history of medicine and for a correct knowledge of the pathogenesis and epidemiology of diseases. These specimens should undergo minimal sampling due to a conservative intent. Modern techniques can be easily performed with satisfactory results with regard to conventional histology and histochemistry, while immunohistochemical methods may be attempted, but their interpretation should be extremely careful, given the high percentage of false negative results.

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DIJAGNOSTIČKA PROCJENA "PLEURALNOG SARKOMA" U DVA MOKRA UZORKA IZ PATOLOŠKE ZBIRKE SVEUČILIŠTA U TORINU

S A Ž E T A K

Patološki muzeji često čuvaju povijesne uzorke sačuvane u tekućini. Posljednjih godina, mogućnost proučavanja takvih primjeraka modernim tehnikama povećala je njihovu važnost zbog njihove biološke i povijesne vrijednosti. Patološka zbirka u Torinu čuva oko 300 mokrih uzoraka koji datiraju iz kasnog 19. i početka 20. stoljeća. Većina ih je još uvijek u izvornim posudama, s naljepnicama na kojima je navedena godina i dijagnoza. Dva slučaja iz ove zbirke označena su kao "pleuralni sarkom" i datiraju iz godina 1896. i 1898. Nalaz izvorne obdukcije nalazi se u knjižnici Instituta za patologiju u Torinu. U današnje vrijeme primarni sarkom bi predstavljao generičku dijagnozu i izuzetno rijedak entitet na pleuri, gdje je najčešća primarna neoplazma maligni mezoteliom. Stoga je provedena ponovna dijagnostička procjena, uključujući rutinsku histopatologiju i imunohistokemiju, kao i arhivski pregled izvornih izvješća. Morfološka analiza i dostupni klinički korelati omogućili su reklasificiranje ovih slučajeva kao metastatskog karcinoma skvamoznih stanica iz nepoznatog primarnog izvora i pleuro-plućnih metastaza leiomiosarkoma maternice. Ova studija sugerira da je krajem 19. stoljeća post mortem dijagnoza pleuralnog sarkoma obuhvaćala različita patološka stanja, često uključujući i sekundarne lezije.