Histopathology and DNA evaluation of wet specimens from the Pathology Collection of Turin

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The authors declare no conflict of interest
The Pathology Collection of Turin houses around 300 wet specimens dating back to XIX-XX century.
Most of these specimens are in their original jars with labels describing year, necropsy number and original old diagnosis.
Due to their original conditions these specimens are an actual **biological archive** and may represent a valid source for research on molecular features of ancient diseases.
Four cases originally diagnosed as lung cancer, uterine myosarcoma, pleural sarcomas (2) underwent modern diagnostic revision and DNA evaluation by conservative sampling.
Case 1: Lung cancer
Case 2: Uterine Myosarcoma
Case 3: Pleural sarcoma (1896)
Case 4: Pleural sarcoma (1898)
Samples from the specimens were submitted to routine histology, histochemistry and immunohistochemistry.

A research was carried out on autopsy report to identify autopsy findings and clinical history.
The revised diagnoses were:

- necrotic lung carcinoma
- uterine leiomyosarcoma
- lung metastases from squamous carcinoma of unknown primary
- lung metastases from uterine leiomyosarcoma
Lung metastases of squamous carcinoma of unknown primary
Reticular fibers staining
Alcian blu staining
Lung metastases from uterine leiomyosarcoma
Trichrome stain
Cytokeratin MNF116
Additional tiny samples underwent DNA extraction and analysis by spectrophotometry and electrophoretic run in agarose gel.

As the chemical composition of the storage fluids is unknown, pH value was measured in each specimen.
pH values were

- **2.56** necrotic lung carcinoma
- **3.15** uterine leiomyosarcoma
- **4.45** lung metastases from squamous carcinoma of unknown primary
- **4.65** lung metastases from uterine leiomyosarcoma
The samples were taken by conservative method and cryostatic sections (-20 degrees C) were obtained 10 microns thick.

Digestion of the section with solution of 75 mM NaCl, 10 mM tris, 0.5 mM EDTA to pH 8.0 and 100 ml of K Proteinase solution. (18 mg/ml)
The samples were incubated at 56 degrees C for 48 hours.

50 ml of fresh K Proteinasei solution was added for 72 hours.

400 ml of solution was then extracted (magnetic beads - Roche MAGNA PURE COMPACT instrumentation).
DNA quantity were assessed using the entire absorption spectrum (220/340 nm) obtained from the Nanophotometer P 300 spectrophotometer.

The concentration of DNA in ng/ml and absorption at 260/280 nm were evaluated on 4 ml samples.
Gel electrophoresis
agarose at 1.3%
RESULTS

The first two samples gave negative results on both spectrophotometric and electrophoretic analysis.
Case 3 – pH 4.45

spectrophotometric analysis absorbance 1.53 6ng/ml

Electrophoretic analysis DNA band with molecular weight about 400bp
Caso 4 - pH 4.65

Spectrophotometric analysis absorbance 1.50 7ng/ml

Electrophoretic analysis DNA band with molecular weight about 500bp
To assess DNA integrity, short tandem repeat (STR) analysis with PowerPlex 16 HS (PROMEGA) panel employed for personal identification was used.
The amplification of amelogenin STRs of chromosome X demonstrates the probable presence of a female subject in case 3.
This allowed us to identify the most probable autopsy report.
CONCLUSIONS

This study confirms the importance of Pathology Collections as a historical archive of diseases that no longer exist or with natural course unmodified and also as biological archive.
Pathology Museums must be preserved as a precious historical and biological heritage!!
Thanks for your attention!