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Review

Thyroid cytopathology: updates and molecular testing

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Summary
The utility of fine needle aspiration (FNA) is well described in the context of evaluating thyroid lesions. Among the various international systems of classification of thyroid cytology, the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has also provided a sound framework to standardize the reporting of FNA cytology results. New molecular evidence and clinical studies demonstrated the need for revision of the nomenclature resulting in introduction of new categories, such as the noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP). Indeterminate thyroid cytology results pose a challenge for further management and the continued development of molecular markers may aid in the management of indeterminate thyroid lesions.

Key words
Fine needle aspiration • Noninvasive follicular thyroid neoplasms with papillary-like nuclear features • Atypical cytology • Molecular tests

Fine needle aspiration (FNA) cytology is important tool in diagnosing thyroid lesions. Ultrasound guided thyroid FNA is usually indicated in thyroid nodules greater than 1.0 cm with high risk imaging features, nodules greater than 2.0 cm with intermediate imaging features or with low risk imaging features but continuously increasing size, or if there is a positive family history of thyroid cancer. Paratracheal lesions or enlarged lymph nodes also requires work up which may possibly include ultrasound guided FNA (US-FNA). The Bethesda System for Reporting Thyroid Cytopathology (TBSRTC) has introduced a uniform system of reporting thyroid FNA cytology results. This system includes six defined categories, but it is comparable to other international systems, such as, the Royal College of Pathology (United Kingdom) system and the Italian consensus 2014. These systems describe five major categories with further subdivision of category 3 into either low risk or atypia and high risk or suggestive of follicular neoplasm. The TBSRTC essentially classifies these subcategories as category III and IV, respectively. Below is a list of the TBSRTC categories and their descriptions:

a. nondiagnostic;
b. benign;
c. atypical (atypia of undetermined significance/ follicular lesion of undetermined significance);
d. suspicious for follicular neoplasm/follicular neoplasm;
e. suspicious for malignancy;
f. malignant.

I. Nondiagnostic

The criteria for adequacy are the presence of six clusters of follicular cells consisting, ideally, of a minimum of 10 cells on one slide. Of note, this criterion is not required in colloid nodules with abundant colloid, lymphocytic thyroiditis consisting of lymphocytes with few or rare follicular cells, or inflammatory/infectious conditions presenting with any atypical cells. Lack of any of the above described adequacy or diagnostic features suggests a nondiagnostic sample (Fig. 1A). Additionally, due to the risk of cystic papillary carcinoma of the thyroid, samples of cystic fluid with less
than six groups of follicular cells are also considered nondiagnostic. Finally, obscuring or air-drying artifacts which affect the diagnostic interpretation are classified as nondiagnostic. False negative FNA results can be avoid by sampling cystic and solid portions of a cyst and by targeting the periphery of larger nodules to avoid central areas of cystic degenerative changes. If FNA is nondiagnostic and imaging or clinical features are suspicious, then repeat FNA is suggested.

II. Benign

This category includes benign colloid nodules (Fig. 1B), lymphocytic thyroiditis, papillary hyperplasia, Graves' disease, and benign cysts. Benign nodules usually consists of mostly macrofollicles mixed with a few microfollicles and abundant colloid. Hurthle cell changes can be seen in large degenerative nodules. Atypia in Hurthle cell nodules or change is an unreliable feature. Treated cases of Graves' disease can show nuclear overlapping or more microfollicles. Nodules with benign cytology results but either continuously increasing in size or with high risk imaging features may require repeat ultrasound guided FNA. However, cases with stable nodular lesions may need imaging follow-up after 24 months in appropriate clinical settings. If repeat FNA is benign in clinically stable nodule then no ultrasound surveillance is required.

III. Atypia

Atypia can be further subclassified into the following categories:

A. Atypia of undetermined significance

This category includes features of cytologic atypia (Fig. 1C), such as, nuclear clearing, nuclear enlargement, sparsely cellular specimens with rare atypical cells, and focal cytologic atypia in lymphocytic thyroiditis. Sometimes, “histiocytoid cells” with focal atypia are seen in cystic papillary thyroid carcinoma (PTC) and they do not show classical nuclear features of PTC. These “histiocytoid cells” are difficult to differentiate from histiocytes. In such instances, immunohistochemical stains can be performed on the cell block material, if available.

Fig. 1. (A) Nondiagnostic: Fragment of muscle (somewhat resembles colloid) (Papanicolaou stained smear) x 20, (B): Benign: Abundant colloid (Diff Quik smear) x 10, (C) AUS: Single atypical cell with nuclear inclusion (cell block) x 40, (D) Suspicious for follicular neoplasm/follicular neoplasm: Cellular specimen with microfollicles and abundant single cell with eccentrically located nuclei (Papanicolaou stained smear) x 10.
b Follicular lesion of undetermined significance

This category includes specimens with architectural atypia. Examples include small or inadequate specimens mostly exhibiting microfollicular architecture or Hurthle cell populations or primarily cellular specimens with a predominance of microfollicular architecture.

c Other

Atypia other than the aforementioned categories fall into this category. Additionally, cases previously treated with radioactive iodine and samples with lymphocytic proliferation or psammomatous calcifications fall here.

The overall risk of malignancy (ROM) is variable in this category, ranging between 6-30% \(^5\,\,^6\). However, it is important to recognize the institutional risk of malignancy. Categorizing architectural atypia separately from nuclear atypia may impact risk of malignancy. Recent study has suggested more risk of malignancy in cases with nuclear atypia (up to 33.3%) than architectural atypia (up to 7.7%) \(^7\). A conservative approach with follow-up is suggested for small and favorable lesions (e.g. those without family history). Repeat FNA will classify a majority of the atypical lesions into a definitive category \(^6\). According to the American Association of Clinical Endocrinologist and American College of Endocrinology guidelines for clinical practice and management, molecular testing may be helpful as an adjuvant test. If repeat FNA or molecular testing is inconclusive, then either surveillance or diagnostic surgery should be considered. Second opinion from a cytopathologist at a high-volume practice may be helpful in certain cases \(^1\).

IV. Suspicious for follicular neoplasm/follicular neoplasm (SFN/FN)

This diagnostic category consists of cellular aspirates with microfollicular architecture, scant or absent colloid, or exclusively Hurthle cell populations either in trabecular architecture or as single cells (Fig. 1D). Cystic changes are usually not seen unless a neoplastic nodule is large, which may allow for degenerative changes. If the neoplastic lesion is comprised of more than 75% Hurthle cells, according to WHO guidelines, it can be designated as a Hurthle cell neoplasm. Parathyroid lesions should be taken into consideration while investigating microfollicular architecture and correlation with clinical history is helpful. The risk of malignancy reported in this category ranges from 25 to 40% \(^3\). Repeat FNA or core needle biopsy is unnecessary. Surgical management, via thyroid lobectomy and isthmectomy, is a preferable option. If the lesion is clinically favorable with low risk imaging features, then close follow-up may be considered. Molecular testing may be helpful in determining the need for surgery.

V. Suspicious for malignancy

Specimens in this category may be cellular with some features of PTC while not meeting the criteria for the diagnosis of carcinoma. This category also includes sparsely cellular specimens with most of the features of papillary thyroid carcinoma. Medullary carcinoma specimens which are largely cellular and contain monomorphic single cells with eccentrically located nuclei and smudged chromatin may fall into this category if they lack cell block which would allow for further characterization. Lymphoma and metastatic tumors should also be considered in the differential diagnosis of a lesion suspicious for malignancy. A surgical approach is recommended and the preferable treatment modality in most of the cases. Repeat FNA for additional sample or flow cytometry can be performed in cases concerning for lymphoma.

VI. Malignant

This diagnostic category includes carcinoma, lymphoma, other tumors, and metastatic tumors. In conventional papillary thyroid carcinoma (PTC), the specimen consists of cells arranged in papillae and/or monolayer sheets with a syncytial appearance. They show intranuclear cytoplasmic pseudo-inclusions, nuclear crowding, intranuclear grooves, and pale nuclei with powdery chromatin (Fig. 2). If the tumor lacks papillae and contains mostly follicular architecture with nuclear feature of PTC, then differential diagnosis should include papillary thyroid carcinoma, follicular variant (PTC-FV) or noninvasive follicular thyroid neoplasms with papillary-like nuclear features (NIFTP). In the latter category, the nuclear changes are mostly subtle and therefore the category “suspicious for follicular neoplasm” or “suspicious for malignancy” can be used. Surgical treatment is recommended management in most cases.

Recently, the NIFTP category was introduced in the thyroid tumor classification replacing encapsulated noninvasive papillary carcinoma follicular variant (NI-EFV-PTC). Nikiforov et al. compared 109 cases (with tumor size 1.1 cm-9.0 cm) of NI-EFV-PTC compared to 101 cases (0.6-5.5 cm) of invasive EFV-PTC. With
a mean follow-up of 14.4 years, the authors did not find any cases of NI-EFV-PTC with any recurrence or metastasis. However, 12 cases with either metastasis or/and recurrence were noted in the comparison group (IEFV-PTC). The most frequent mutation in the NI-EFV-PTC was RAS, a commonly mutation seen in follicular adenoma (FA). These findings supported an indolent behavior for NI-EFV-PTC which resulted in recommendation for a nomenclature shift to NIFTP. Johnson and colleagues found RAS mutation in FA (4/11) and NIFTP (20/32) but no cases in PTC with extensive follicular growth pattern and suggested molecular similarities between FA and NIFTP.

Specific histologic criteria are required to establish a diagnosis of NIFTP (Fig. 3). Neoplastic cells should be in microfollicular architecture, show some nuclear features of PTC, and either encapsulated or with demarcated outline and no capsular or vascular or intraparenchymal invasion noted. NIFTP usually show some nuclear features including irregular nuclear membrane, nuclear enlargement, and chromatin clearing. Intranuclear pseudoinclusions are very rare and infrequently seen. No papillae, psammoma bodies, necrosis, or increased mitosis (> 3), or solid growth > 30% should be seen. NIFTP cases are BRAF600E and TERT negative and they usually show indolent behavior and do not metastasize. In the original study suggesting NIFTP classification, all cases of NI-EFV-PTC cases were larger than 1.0 cm which raises concerns about the classification of lesions less than 1.0 cm as encapsulated/well circumscribed PTC (microPTC versus NIFTP).

Another group compared 52 cases of encapsulated follicular variant of microPTC (EFV) and 57 invasive EFV-microPTC and followed the cases for two years. They did not find any recurrence in the first group (noninvasive) but found 5 cases with lymph node metastasis in the invasive EFV-PTC group. They concluded the study with suggestion to include noninvasive EFV-PTC in NIFTP category.

Cytologic evaluation of NIFTP cases poses a challenge when differential diagnosis includes PTC-FV. Most of the studies proposed classifying NIFTP as Bethesda category IV (suspicious for follicular neoplasm/ follicular neoplasm) or Bethesda Category V (suspicious for malignancy) with a disclaimer about

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**Fig. 2.** Papillary carcinoma, (A) Clusters of papillary carcinoma with focal nuclear crowding x 10 (Diff-Quik smear), (B) Elongated and oval nuclei, nuclear overlapping and intranuclear inclusion (arrow) Papanicolaou stained smear x 40, (C) Fragment of papillary carcinoma (cell block) x 10, (D) Tissue section with focus of papillary carcinoma x 20.
the differential diagnosis. Cytomorphologic features of NIFTP usually show microfollicular architecture and some nuclear features of PTC with rare pseudo-inclusions \(^{11}\ 12\). On the other hand, they show RAS, BRAFK601, PPARG, and THADA gene fusions while being negative for BRAF600E. Due to the classification of NIFTP as an indolent neoplasm rather than malignant carcinoma, one would expect a decrease in risk of malignancy in indeterminate cytology cases. Bethesda classes III and V showed a decrease in the observed risk of malignancy (Bethesda III: 10-30% to 6-18% and Bethesda V: 50-75% to 45-60%), while the risk of malignancy in category IV remained comparable \(^{13-15}\). This new understanding of the risk of malignancy of NIFTP raises questions about the use of lobectomy versus total thyroidectomy in future investigations and the use of molecular testing as an aid in determining the surgical approach. Molecular markers are developed to guide further management in indeterminate thyroid lesions. Molecular tests can either be a rule in test or rule out test. A rule in test is used to predict malignancy and helps guide surgical management. They usually have a high positive predictive value (PPV) and specificity (confirmatory test). Rule out tests helps identify cases without disease and thereby avoiding unnecessary surgical treatment in patients with indeterminate cytology results. Rule out tests should have high negative predictive value (NPV) and sensitivity. There are some challenges associated with the use of molecular testing such as cost, insurance coverage, and collection and preservation of the sample. Currently, there are four clinically used molecular tests and they are described and listed below:

1. Afirma Gene Expression Classifier;
2. Thyroseq Genomic Classifier;
3. ThyGeNEXT and ThyraMIR;
4. RosettaGX.

1. Afirma Gene Expression Classifier

This is a DNA microarray-based test used for intermediate cytology results. Results are reported as BENIGN or SUSPICIOUS \(^{16}\). Initial FNA passes are utilized for cytologic diagnostic evaluation while two sep-
perate FNA passes are stored in a vial with nucleic acid preservative. The initial screening is performed with 25 genes, including those genes for metastatic tumors, parathyroid, and medullary carcinomas (MTC). If the latter is positive, it is reported as a positive result. If the initial screen is negative, then the specimen is examined for an additional 142 genes to complete the main GEC panel. Benign results are low risk (5%) while suspicious results are considered moderate risk (40%). If BRAF 600E is positive then the specimen is considered high risk (100%).

McIver et al. in a study of 105 indeterminate cytology cases evaluated Afirma GEC results in 36 cases and compared the ROM using histologic follow-up. Of the 32 GEC-suspicious cases, 5 were found to be malignant while 27 were benign. They reported statistical results (% sensitivity, specificity, PPV, and NPV) as follows: 83, 10, 16, and 75, respectively. In one of the initially published studies of 265 indeterminate cytology results including AUS/FLUS (n = 129), SFN/FN (n = 81), and suspicious for malignancy (n = 55), the authors found Afirma GEC analysis of AUS/FLUS to have the following results (% sensitivity, specificity, PPV, and NPV): 90, 53, 38, and 95, respectively. For SFN/FN, the results (% sensitivity, specificity, PPV, and NPV) were as follows: 90, 49, 37, and 95, respectively.

In instances with indeterminate cytology results with negative Afirma GEC testing, it is appropriate to follow-up as if the lesions were benign. Azizi et al. evaluated 151 indeterminate cytology results and found GEC suspicious results in 59 and GEC benign in 92 cases. Of the GEC-suspicious group, 28 (47.5%) were malignant while 17 cases in the benign group received follow-up surgery and 3 (3.3%) of these cases were found to be malignant on follow-up histology.

2. THYROSEQ Genomic Classifier

This method utilizes DNA and RNA next generation sequencing to detect hot spot mutation, gene fusions, gene alterations including copy number variations (CNV) with ThyroSeq v3. Currently, ThyroSeq v2 (2014) uses a 56 gene panel and Thyroseq v3 (2017) has 112 gene panels available for clinical use. This test is reported as either NEGATIVE or POSITIVE. Specimen collection requires either a separate FNA pass into Thyroseq Preserve solution or formalin fixed paraffin embedded tumor sections. A recent study evaluated 247 indeterminate cytology results (Bethesda III and IV) and compared them to corresponding Thyroseq v3 and histology (cancer + NIFTP) results. They found overall sensitivity to be 94%, specificity 82%, PPV 66%, and NPV 97%. For Bethesda category III, the different variables (%; sensitivity, specificity, PPV, and NPV) for Thyroseq v2 and v3 are compared as follows: 91, 92, 77, 97 and 91, 85, 64, 97, respectively.

3. ThyGeNEXT and ThyraMIR

This method requires DNA and RNA extraction by next generation sequencing (NGS). A dedicated FNA pass is required and the specimen is preserved in fixative (up to six weeks in a room temperature). ThyGeNEXT NGS Panel consists of DNA mutation panel (BRAF, PIK3A, HRAS, KRAS and NRAS) and chromosome rearrangements (RET-PTC1, RET-PTC3 and PAX8-PPARG). If initial testing with ThyGeNEXT shows either BRAF or TERT mutations, this is considered high risk and surgical options should be considered. If ThyGeNEXT shows other mutations, then ThyraMIR consisting of 10 miRNA (miR-29b-1-5p, miR-31-5p, miR-138-1-3p and others) is the next step and the result is reported as LOW or MODERATE risk. Labourier et al. studied 109 indeterminate cytology results (Bethesda III & IV) and found (% sensitivity, specificity, PPV and NPV as follows: 94, 80, 68, 97 and 82, 91, 82, 91, respectively.

4. RosettaGX

This test measures 24 miRNA by using quantitative RT-PCR. Some of the miRNA overlap with ThyraMIR® miRNA classifier (hsa-miR-31-5p, hsa-miR-222-3p, hsa-miR-146b-5p, hsa-miR-375, hsa-miR-551b-3p). Testing requires cell acquisition directly from the ThinPrep slide and direct smears and results are reported as BENIGN or SUSPICIOUS. An initial validation study evaluated indeterminate thyroid cytology results (Bethesda III & IV) by dividing them in two categories; validation set (n = 189; when one out of two pathologists agree with the original pathologists diagnosis) and validation agreement set (n = 150; when all three pathologists agree with the diagnosis). They found (% sensitivity, specificity, PPV and NPV in these two groups as follows: 85, 72, 59 and 91, 78, 62 99, respectively.

According to the American Thyroid Association management guidelines, molecular testing should be performed in Clinical Laboratory Improvement Amendments (CLIA)/College of American Pathologist certified laboratories. In Bethesda III cases, repeat FNA and molecular testing may help in ROM assessment and should be performed based on clinical judgment and patient preferences. If repeat FNA or molecular
testing is inconclusive, then either surveillance or diagnostic surgery should be considered. On the other hand, in Bethesda category IV, surgical management is usually performed while molecular testing may be used to assess the ROM 24.

CONFLICT OF INTEREST STATEMENT
None declared.

References

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Original Article

When tumor doesn’t read textbook.
Third case of TTF1 and p40 co-expression in the same tumour cells in a non-small cell carcinoma. A potential new entity to consider?

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Summary
Introduction. The 2011 WHO Classification for lung adenocarcinoma enlightened the need for a wise use of immunohistochemistry to preserve tissue for both diagnosis and molecular studies. The current recommendation is to use a panel comprising TTF1 and p40 to classify tumors with no clear squamous or glandular differentiation as many studies have showed the higher specificity of p40 over p63 as marker of squamous differentiation. However, the co-expression of both markers opens a new scenario with subsequent classification and potentially treatment issues.

Materials and methods. We report a case of a non-small lung cell carcinoma (NSCLC) with coexistent expression of TTF1 and p40 in the same tumour cells. To our knowledge, this peculiar immunohistochemical profile is very rare, and thus a review of the clinical and molecular features including molecular variances of the tumour was performed. Review of the pertinent literature was also carried out.

Results. Two additional articles describing unusual cases of NSCLC with coexistent expression of TTF1 and p40 were found and compared to our case. Interestingly, they all carried out aberrant mutation in TP53 oncogene and were of advance stage.

Conclusion. The positivity for both “squamous” and “adenocarcinomatous” markers and mutations of TP53 could be the expression of a not fully recognized variant of NSCLC with possible implications for classification, diagnosis and therapy.

Key words
Lung squamous cell carcinoma • Lung adenocarcinoma • TP53 mutations • TTF-1 • P40

In 2011 the International Association for the Study of Lung Cancer (IASLC), the American Thoracic Society (ATS) and the European Respiratory Society (ERS) have jointly proposed a new classification for lung adenocarcinomas. This classification, which also includes molecular features, stressed the need to optimize the management of the tissue available in order to render both diagnosis and molecular studies.

An algorithm based on morphological and immunohistochemical features recommends thyroid transcription factor-1 (TTF1) and p63 as markers for adenocarcinoma (ADC) and squamous cell carcinoma (SCC) respectively. Recently p40, an isoform of p63, has shown greater sensitivity and specificity in identification of SCCs when compared to p63. Bishop et al. demonstrated that although p40 and p63 have the same sensitivity, polyclonal p40 has higher specificity as p63 antibody can stain up to 20-30% of ADCs leading to confusion in some poorly differentiated tumors. The recommendation is to use a panel comprising TTF1 and p40 which are generally mutually exclusive to classify tumors with no clear squamous or glandular differentiation and with solid/pseudosquamous histology. In fact, they can be misclassified as SCCs with severe treatment implication: the exclusion from molecular testing and potentially lethal pulmonary hemorrhage in patients treated with bevacizumab.

However, Pelosi in 2015 and Hayashi in 2018 have both described unusual cases of NSCLC with co-expression of TTF1 and p40 in the same cells. Interest-
ingly both cases have a similar molecular signature harboring TP53 mutation. We reported the third case with similar features.

A 51-years-old male smoker patient (the number of cigarettes was not specified) was referred to our hospital for persistent headaches and a head RMI revealed multiple metastases in his brain. A 3.1 cm lung mass with associated mediastinal lymphadenopathy and adrenal lesion was subsequently discovered on CT scan and biopsy was performed (stage cT2a N3 M1c). The core of lung tissue showed a NSCLC with morphology slightly favoring squamous differentiation with occasional intercellular bridges and dense eosinophilic cytoplasm. Tumor cells showed strong and diffuse positive staining for p40 (Diagnostic BioSystem), TTF1 (Clone SPT24 Leica Biosystem Newcastle) and Napsin-A in the same tumor cells (Fig. 1). Our clone was SPT24 ready to use, while the other reports used the other clone so this means that more than one antibody highlights these type of tumours. In view of the positivity for adenocarcinoma markers, the sample was sent for molecular testing, ALK (negative) and PD-L1 (strong positive) testing. ALK was analyzed by immunohistochemistry using VENTANA ALK (D5F3) CDx Assay intended for the qualitative detection of the anaplastic lymphoma kinase (ALK) protein in formalin-fixed, paraffin-embedded (FFPE) non-small cell lung carcinoma (NSCLC) tissue stained with a BenchMark XT or BenchMark ULTRA automated staining instrument. It is considered positive if there is presence of strong granular cytoplasmic staining in tumor cells (any percentage of positive tumor cells). Positive control used was the presence of strong granular cytoplasmic staining in ganglion cells in appendix. PDL1 was analyzed by immunohistochemistry using VENTANA PD-L1 (SP263) Assay intended for the qualitative detection of the programmed death ligand 1 (PD-L1) protein in formalin-fixed, paraffin-embedded (FFPE) NSCLC tissues stained with OptiView DAB IHC Detection Kit on a BenchMark IHC/ISH instrument. It was considered positive if there is presence of any amount of membranous staining in tumor cells of any intensity (percentage of positive tumor cells and their intensity recorded). Positive control used was the presence of membranous staining in placenta. ROS-1 was not tested.

Fig. 1. (A) Lung biopsy showing a non-small cell carcinoma with predominantly solid pattern and no clear glandular differentiation or keratin formation (H&E, 10X). (B) Close up of the tumor featuring abundant eosinophilic cytoplasm and pleomorphic nuclei, hint of intercellular bridges is questionable (H&E, 20X). (C) Tumour cells show strong nuclear staining for TTF-1 (10X). (D) Strong nuclear immunoreactivity for p40 in the same tumor cells. The decoration pattern is identical for both markers (10X).
Mutational screening was performed by next generation sequencing using the Ion Torrent Cancer Hotspot panel v2. This assay comprises 207 amplicons in 50 onco-genes frequently mutated in solid tumors. DNA was extracted from paraffin embedded tissue using the Qiagen QIA symphony DSP DNA Mini Kit. The results showed no actionable mutations but a polymorphism in TP53 (TP53)c.215C > G (p.Pro72Arg) which is currently associated with inherited cancer predisposition syndrome. These features suggests that there is a still small but significant group of NSCLS with coexpression of TTF-1 and p40 in the same cells; in order to further characterize these tumors and best classify them as more comparable to adenocarcinomas or squamous cell carcinomas, we suggest a panel of markers including TP53, Napsin-A, ALK and PD-L1. The new WHO classification of lung cancer contains recommendations to provide the most accurate diagnosis for every type of sample (biopsy, resection or cytology). The need to identify non-small/non-squamous carcinoma has become essential in order to test these cases for actionable mutations. The more squamous-specific marker p40 has been used in few studies to correctly re-classify solid/pseudosquamoid tumors showing co-expression of TTF1 and p63 3. However, these three cases open a new horizon for identification and classification of peculiar multi-phenotypic tumors. Pelosi in 2015 4, reported an “amphicrine” biphenotypic tumor on a lung biopsy. The biop-sy contained a high grade NSCLC with focal areas suggestive for squamous differentiation. Immunohis-tochemistry showed strong and diffuse positivity for both p40 and TTF-1 and electronic microscopy confirmed these features. The lesion also showed a TP53 mutation and a gene amplification of FGFR-1. In 2018 Hayashi et al. reported a case of NSCLC with strong and diffuse positivity for p40 and TTF-1 together with mutations on the allelic DNA for TP53 and PTEN genes. The authors speculated that mutations in the key genes such as TP53, PTEN, FGFR-1 and others would promote the selection of peculiar stem cells leading to poorly differentiated and multi-phenotypic tumors. TP53 is commonly mutated in many tumors 9. TP53 executes its tumor-suppressive phenotype through controlling the transcription of many target genes in response to stress signals such as DNA damage, environmental hazards, toxins and oncogene activation. The mutated TP53 loses its oncosuppressor function.

Using in vitro and in vivo models, Jeong et al. in 2017 7 demonstrated that the deletion of TP53 in tracheal epithelial cells promotes self-renewal and development of tumor cells with features similar to squamous cell carcinoma, while the same deletion in peripheral lung cells lead to adenocarcinoma-like cell formation. The type of lung cancer formed depends on the cell type targeted by deletion of TP53. Mutations of TP53 occur frequently in NSCLC: over 75% of SCCs and over 55% of ADCs 8. Lung ADCs not harboring TP53 mutations usually show that the gene is altered by ubiquitination – which leads to its degradation – and/or accumulation. Even wild-type TP53 can play a role in the development of ADCs with no evidence of mutations 9. Clinically, mutations of TP53 are associated with higher tumor size, stage and lymph node metastases 10. The patient of the present case underwent whole brain radiotherapy followed by chemotherapy and showed a partial reduction of size of brain metastases, but the disease is progressing rapidly, and the patient is deteriorating at the time of writing.

| Tab. I. Non-small lung cell carcinoma with co-expression of TTF-1 and p40: cases reported in literature. |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Gender/age | Smoking history | Imaging | Histology | IHC | Molecular |
| Pelosi 2015 | Male/ 77yrs | Ex-smoker (40 pack year) | Left hilar tumour (85mm) | High grade NSCLC with hints of squamous differentiation | P40 (clone BC28 Biocare Medical Concorde CA) and TTF1 (clone 8G7G3/1, Dakopatts, Glostrup, Denmark) positive |
| Hayashi 2018 | Male/ 73yrs | Ex-smoker (141 pack year) | Left upper lobe tumour (19mm) | NSCLC with hints of glandular differentiation and areas negative for mucin stain | P40 (clone BC28 Biocare Medical Concorde CA) and TTF1 (clone 8G7G3/1, Dakopatts, Glostrup, Denmark) positive |
| Present case | Male/ 51yrs | Current smoker | Right upper lobe tumour (31mm) | High grade NSCLC with hints of squamous differentiation | P40 (Diagnostic Biosoystem RP163-05) and TTF1 (Bond ready to use primary antibody clone SPT-24, Leica biosystem, Newcastle Ltd) positive |

* (*p*Val272Leu) (TP53)c.215C>G (p.Pro72Arg)
In general, since patients with lung carcinomas featuring TP53 mutations have poorer outcomes, the gene may be used as prognostic marker in clinical practice and could also represent a target for cancer molecular therapy.

At present IASLC, ATS and ERS guidelines do not mention the co-expression of both markers 40 and TTF-1 in the same tumor cells. The case hereby presented, together with the two previously reported, has this peculiar immunohistochemical profile associated with an allelic mutation of oncosuppressor gene TP53. We could speculate that these combined features – positivity for both “squamous” and “adenocarcinomatous” markers and mutations of TP53 could be the expression of an aggressive, not yet recognized variant of lung adenocarcinoma or adenosquamous carcinoma which could be considered for further classification, specific diagnostic approach and possibly targeted therapy in the near future.

CONFLICT OF INTEREST STATEMENT
None declared.

References

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Stromal IL2 is related to the neutrophil/lymphocyte ratio in epithelial ovarian cancer

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Introduction. There is a need for the development of new biomarkers for diagnosis and prognosis of ovarian cancer, which can ideally serve as targets for new therapeutic modalities and individualization of treatment. The objectives of this study were to determine the prognostic significance of the neutrophil/lymphocyte ratio in the peripheral blood of patients with ovarian cancer and tumor staging, and to associate this marker with the immune expression of a panel of cytokines.

Methods. The study included 24 patients with malignant ovarian neoplasia treated at the Pelvic Mass Outpatient Clinic of the Clinical Hospital of the Federal University of Triângulo Mineiro. The neutrophil/lymphocyte ratio was calculated as the absolute number of neutrophils divided by the absolute number of lymphocytes. Expression of the cytokines was evaluated by the immunohistochemistry method (IL2, IL5, IL6, IL8, IL10 and TNF-R1). Fisher’s statistical test was used for the comparisons of immunohistochemical expression with the neutrophil/lymphocyte ratio, and the unpaired T-Test was used in the analysis of the association of this ratio with tumor staging.

Results. A neutrophil/lymphocyte ratio > 2.6 was significantly higher in the more advanced stages (II-IV) of malignant ovarian neoplasia (p = 0.0098). In addition, this ratio > 2.6 was associated with IL2 stromal immunostaining (1-3) (p = 0.0472).

Conclusion. Stromal IL-2 is associated with a higher neutrophil/lymphocyte ratio, suggesting a worse prognosis in ovarian cancer and its role in tumor immunology; a neutrophil/lymphocyte ratio > 2.6 is associated with more advanced stages of malignant ovarian neoplasia.

Key words
Neutrophil/lymphocyte ratio • Staging • IL-2 • Immunohistochemistry • Ovarian cancer


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Introduction
Ovarian cancer is one of the types of gynecological tumors that cause the most deaths worldwide, presenting a low overall survival rate. It is an insidious disease and is commonly diagnosed in more advanced stages. The American Cancer Society estimates that about 22,440 women will have a new diagnosis of the disease in the United States by 2017, and about 14,080 will die of disease. Ovarian cancer has a strong association with inflammation, and it is well known that inflammation is associated with different stages of tumor development, including initiation, promotion, malignant conversion, invasion and metastasis appear to be regulated by cytokines. Therefore, several systemic inflammatory markers may have potential applications in the prediction of poor prognosis.

In recent years, laboratory quantification of systemic inflammatory response markers such as C-reactive protein (CRP), absolute leukocyte count, neutrophil/lymphocyte ratio (NLR) and platelet/lymphocyte ratio (RPL) were introduced as prognostic factors in patients with various types of malignant neoplasms, including ovarian cancer. Studies involving the neutrophil/lymphocyte (NLR) ratio as an inflammatory marker have shown favorable results, suggesting that it can be used as a significant predictor of malignancy for solid tumors originating from various tissues. There is a need for the development of new biomark-
ers for diagnosis and prognosis of ovarian cancer, which can ideally serve as targets for new therapeutic modalities and individualization of treatment.  

The objectives of this study were to determine the prognostic significance of NLR in ovarian cancer patients, and to verify the stromal expression of cytokines (IL2, IL5, IL6, IL8, IL10 and TNF-R1) in malignant ovarian neoplasia.

Materials and methods

We prospectively evaluated 24 patients with malignant ovarian neoplasia treated at the Pelvic Mass Ambulatory of the Discipline of Gynecology and Obstetrics/Oncology Research Institute (IPON), Federal University of Triângulo Mineiro - UFTM, submitted to surgical treatment according to established criteria, from 2009 to December 2013 and with subsequent histological diagnosis of epithelial ovarian cancer confirmed by the pathologist. The criteria for exploratory laparotomy were: anechoic cysts with a maximum diameter of less than 7.0 cm and persistence of alteration for more than 6 months and normal tumor markers; altered tumor markers; anechoic cysts with a maximum diameter greater than or equal to 7.0 cm; ovarian masses with solid contents, presence of intracystic vegetation, thick septa, 2 or more fine septa; Color Doppler with solid contents, presence of intracystic vegetation, thick septa, 2 or more fine septa; Color Doppler with resistance index less than or equal to 0.4.  

The specimens obtained by surgical resection were processed in paraffin and reviewed by an experienced pathologist. The selected cases were submitted to new cuts (4 μm) on silanized slides (ATPS - Silano, Sigma® A3648), using the Novolink™ Polymer Detection System. The slides were kept in a greenhouse (56 °C, 24 h) and then dewaxed (3xylol baths, 5 min each) and dehydrated (three baths of absolute alcohol and one 80% alcohol bath, 10 sec each). After, the slides remained in the bath (PBS, pH 7.2, 5 min) for hydration.

Recovery of the antigens was then performed. The slides were placed in cytology tubes containing 10 mM citrate buffer (pH 6.0) or Tris-EDTA, as directed by the manufacturer, and placed into a Pascal pan, which was quenched with distilled water to the limit indicated for 30 minutes at a temperature of 100 °C. Next, the tubes were removed from inside the pan and placed on the bench for its cooling.

To neutralize the endogenous peroxidase the Peroxidase Blocker was used for 5 minutes. Washed in PBS for 5 minutes. Incubated with Protein Blocker for 5 minutes. The slides were washed with PBS for 5 minutes. The sections were incubated overnight at 4°C with a primary antibody.

The primary antibodies used were IL-2 Antibody (H-133; Rabbit polyclonal IgG; sc-7896; Lote #H0811; Santa Cruz Biotechnology, Inc.; Dallas, Texas), IL 5 Antibody (H-85; Rabbit polyclonal IgG; sc-7887; Lote D0708; Santa Cruz Biotechnology, Inc.; Dallas, Texas), NLC-L-IL-6 Antibody (Mouse Monoclonal; Lote L155309; Leica Biosystems Nussloch; Nussloch, Germany), IL-8 RB (E-2) Antibody; Mouse Monoclonal IgG; sc-7304; Lote F1510; Santa Cruz Biotechnology, Inc.; Dallas, Texas), IL 10 Antibody (Polyclonal; Lote 11042807; cat n° 250713; ABBIOTEC; San Diego), p-TNF-R1 (ser 274) Antibody (Rabbit polyclonal IgG; Lote B0509; sc-130220; Santa Cruz Biotechnology, Inc.; Dallas, Texas).

After overnight incubation at 4°C with specific antibody, the slides were placed at room temperature (15 min), washed (PBS) for 5 minutes and dried. Incubated with Primary Powder for 30 minutes. Washed in PBS for 5 minutes. Incubated with Novolink™ Polymer for 30 minutes. Washed in PBS for 5 minutes.

After washing in PBS, the slides were developed by chromogen solution (Diaminobenzidin-DAB) for 5 min. Following this, the slides were washed (running water) and counterstained in Harris hematoxylin. Finally, the slides were immersed in 3 baths of absolute alcohol (10 seconds each), to remove excess water, 1 xylene bath phenicate and 3 xylol baths (5 minutes each). The coverslips were added on the blades with entellan for further analysis.
Positive and negative controls were used. Two observers evaluated the slides. The intensity of immunostaining in the stroma was subjectively assessed using 0 to 3: 0 (no labeling), 1 (weak labeling), 2 (moderate labeling), 3 (strong labeling).

**Neutrophil/Lymphocyte Ratio**

The absolute values of neutrophils and lymphocytes were collected from the preoperative hemogram. NLR values were obtained by dividing the absolute number of neutrophils by the absolute number of lymphocytes. It was used as a cutoff value for this relationship, based on previous studies in patients with ovarian epithelial cancer.

**Statistical Analysis**

The data were analyzed with GraphPad Prism software. Unpaired T-test was used to compare NLR and tumor staging, and Fisher’s exact test was used to compare the cytokines and NLR immunostaining. In the immunohistochemical study, agreement among the three observers was performed through kappa: $\kappa < 0.4$: weak agreement; $0.4 \leq \kappa < 0.8$: moderate agreement; $0, 8 \leq \kappa < 1$: strong agreement; $\kappa = 1$: perfect agreement.

Differences were considered significant at $p < 0.05$. All discordant cases were reevaluated, and the result was defined by consensus. Fisher’s exact test was used, with a level of significance lower than 0.05.

**Results**

The mean age of the patients analyzed was 48.3 (± 14.4) years. The histological diagnosis of the 24 cases of malignant ovarian neoplasms was: 9 (37.5%) borderline mucinous tumors, 8 (33.3%) serous cystadenocarcinomas, 2 (8.3%) mucinous cystadenocarcinoma, 1 (4.2%) borderline serous tumor, 2 (8.3%) adenocarcinomas, 1 (4, 2%) clear cell carcinoma and 1 (4.2%) endometrioid adenocarcinoma (Tab. I).

In relation to the staging, 12 (50.0%) had staging I, 1 (4.2%) staging II, 9 (37.5%) staging III and 2 (8.3%) staging IV. Regarding tumor grade, they were classified in 1, 2 or 3, according to the cell differentiation. Ten (41.7%) patients had histological grade 1, 10 (41.7%) had histological grade 2, and 4 patients had histological grade 3 (16.6%).

NLR > 2.6 was significantly higher in the more advanced stages (II-IV) of malignant ovarian neoplasia ($p = 0.0098$) compared to stage I (Fig. 1). In addition, NLR > 2.6 had association with stromal expression (staining 1-3) of IL2 compared to immunolabeling 0 ($p = 0.0472$) (Tab. II).

There was no statistical significance in relation at other cytokines studied. NLR > 2.6 there was no association with stromal expression (staining 1-3) compared to immunolabeling 0 of IL6 ($p = 0.1049$), IL5, IL8, IL10 and TNF-R1 ($p = 1.0000$).

---

**Tab. I.** Histological diagnosis and histological grade (FIGO) of the malignant and borderline ovarian neoplasms (Raspolini et al., 2007).

<table>
<thead>
<tr>
<th>Malignant and borderline ovarian neoplasms (n = 24)</th>
<th>n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Borderline mucinous tumors</td>
<td>9</td>
<td>37.5</td>
</tr>
<tr>
<td>Grade 1 = 9 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous cystadenocarcinoma</td>
<td>8</td>
<td>33.3</td>
</tr>
<tr>
<td>Grade 2 = 7 (87.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 3 = 1 (12.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous cystadenocarcinoma</td>
<td>2</td>
<td>8.3</td>
</tr>
<tr>
<td>Grade 2 = 2 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>2</td>
<td>8.3</td>
</tr>
<tr>
<td>Grade 2 = 2 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>Grade 3 = 1 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearcell carcinoma</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>Grade 3 = 1 (100%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Borderline serous tumor</td>
<td>1</td>
<td>4.2</td>
</tr>
<tr>
<td>Grade 1 = 1 (100%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Association of NLR > 2.6 with stages II-IV of ovarian epithelial cancer ($p = 0.0098$).

**Tab. II.** Association of stromal IL-2 immunostaining with NLR.

<table>
<thead>
<tr>
<th>IL-2 staining</th>
<th>0</th>
<th>1, 2, 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>NLR &gt; 2.6, 6 (n = 11)</td>
<td>2 (18.2%)</td>
<td>9 (81.8%)*</td>
</tr>
<tr>
<td>NLR ≤ 2.6, 6 (n = 13)</td>
<td>8 (61.5%)</td>
<td>5 (38.5%)</td>
</tr>
</tbody>
</table>

Fisher’s exact test, *$p = 0.0472$.*
Discussion

Ovarian cancer is associated with a low survival rate, and high morbidity and mortality. This is mainly due to late diagnosis and ineffective screening methods. New strategies need to be identified to define new prognostic factors for ovarian neoplasia 19. Prognostic factors defined by correlation with survival generally reflect the extent of disease (stage), intrinsic tumor biology (type and histologic grade), and the patient's ability to tolerate treatment for the disease 20. Inflammatory biomarkers of peripheral blood, such as the neutrophil/lymphocyte ratio, have been used as prognostic markers in solid tumors, including as ovarian tumors 6 9.

Inflammation has been recognized as one of the hallmarks of almost all human cancers. The tumor-related inflammatory microenvironment could facilitate tumor growth and metastasis by sustained proliferation, inhibiting apoptosis, inducing the epithelial-mesenchymal transition, initiating angiogenesis and suppressing host anti-tumor immunity. For epithelial ovarian cancer, epidemiological studies have revealed pelvic inflammatory diseases may increase risk, while inflammation caused by incessant ovulation remains one of the well-accepted hypotheses of its carcinogenesis 6. It is possible to identify an important relation in neutrophil and lymphocyte counts and the risk of patients regarding the complications and poor prognosis in malignant ovarian diseases. This immunological understanding allows new therapies, with prognostic evaluation and improvement in patients' quality of life 8.

In the study by Li et al. (2017) 6, preoperative NLR was elevated in ovarian epithelial cancer, and was significantly associated with characteristics of high tumor burden and advanced stages of the disease. Also, two independent studies with colorectal cancer added evidence to this hypothesis. Chenet al. (2015) 21 indicated that an NLR > 5 was associated with poor prognosis in metastatic colorectal cancer and was correlated with increased expression of inflammatory cytokines, such as interleukin 6 (IL-6), IL-8, IL-2, HGF, macrophage colony stimulating factor (M-CSF), and epidermal vascular growth factor (VEGF). In our study, when comparing peripheral blood RLN in patients with malignant ovarian neoplasia and its tumor staging, the NLR > 2.6 is associated with more advanced stages of malignant ovarian neoplasia (p = 0.0098).

The presence of high levels of IL-2 receptors has been found in the serum of patients with various types of solid tumors 22 23. In malignant ovarian neoplasia, an increase in IL-2 receptor concentrations can be found in more advanced stages and may play a role in predicting the risk of recurrence 24. Serum IL-2 receptor levels have been found to be significantly elevated in ovarian cancer patients compared to benign gynecological tumors and healthy patients, and may reflect the status of the immune system and the severity of the disease, being a possible marker of prognosis 25. Jammal et al. (2016) 26 demonstrated stronger IL-2 immunostaining in malignant neoplasms compared to benign ovarian neoplasms, and there was a strong immunostaining relationship with histological grade 3 in malignant ovarian neoplasms. Our findings are consistent with these studies; stromal immunoeexpression of IL-2 (1-3) was associated with NLR > 2.6.

There was no statistical significance in relation to other cytokines studied. It is important to note that there is a heterogeneous response of the cytokine expression profile, in these pathways may reflect underlying differences in stroma biology and inflammatory response intrinsic to the various sites 27.

The main limitation of the study is the small sample of patients. Other studies with a larger number of patients are needed to clarify the role of IL-2 and other cytokines in the prognosis and progression of ovarian cancer.

Conclusion

Stromal IL-2 is associated with a high neutrophil/lymphocyte ratio, suggesting poorer prognosis in ovarian cancer and its role in tumor immunology; a neutrophil/lymphocyte ratio > 2.6 is associated with more advanced stages of malignant ovarian neoplasia.

Acknowledgements

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Conflict of Interest Statement

None declared.

References


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Peripheral nerve mucoid degeneration involving the sciatic nerve

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Summary
Peripheral nerve mucoid degeneration (PNMD) is a rare non-neoplastic degenerative condition characterized by endoneural deposit of mucoid matrix. Herein, we report a case of PNMD involving the sciatic nerve with preoperative features, surgical treatment and pathological findings.

Key words
Peripheral nerve mucoid degeneration • Sciatic nerve

Introduction
Peripheral nerve mucoid degeneration (PNMD) is a rare, non-neoplastic degenerative condition, characterized by localized axonal damage and Schwann cell/myelin loss associated with intra-neural mucoid changes and fibrosis. PNMD is usually due to chronic nerve injury and entrapment syndrome, and it usually affects the upper extremities, mainly in young adult male patients. Differential diagnosis should be carried out with focal mucoid degeneration, which can be observed as progressive neuro-muscular atrophy (Charcot-Marie-Tooth) or peripheral polineuropathy related to hypothyroidism. In daily practice, PNMD is encountered in small cutaneous nerves entrapped in scars after surgical procedures. Local pain, paresthesias, nerve paralysis and possible palpable swelling are the commonest symptoms. Magnetic resonance imaging (MRI) may help in visualizing alteration of the affected nerve, but MRI features are often non-specific. Therapeutically, surgical neurolysis may improve the clinical picture, avoiding recurrences, but the functional result depends on the integrity of neural fibers. Herein, we report a case of PNMD involving the sciatic nerve, describing preoperative features, surgical treatment and pathological findings.

Case report
A 52-years-old man presented in August 2016 complaining of pain and paresthesia localized in the left ankle, not referred to any injuries or previous trauma. Lumbar radiography did not reveal any pathological features. After a few weeks, the symptoms dramatically increased leading to a complete paralysis of the left leg and foot. The patient was submitted to a MRI of the lumbar spine, which did not show any disk disease or other spinal pathology that could be causing the clinical symptoms. On the other hand, electromiography and neurography studies showed complete mono-neuropathy of the left sciatic nerve, mainly in the distal part of peroneal and tibial compartments, with both sensory and motor impairment. Therefore, MRI was repeated with contrast medium,
focusing on the pelvis. This new MRI showed a diffuse and regular enhancement, measuring 8 cm in length, along the left sciatic nerve, involving the region below the piriform muscle (Fig. 1).

The sciatic nerve did not show features of neoplastic swelling, although the signal intensity suggested the presence of an intra-neural lesion. The MRI features could not rule out benign or malignant neoplasms and therefore a surgical exploration was delivered.

On admission, the patient exhibited a complete left sciatic nerve paralysis in the left leg and foot, with evoked pain by palpation in the rear side of thigh and sensory alteration in the anterior-lateral side of leg and the dorsum of the foot.

The patient was operated on in the prone position, with a surgical approach to the left sciatic nerve through the gluteus. On surgical exploration, the sciatic nerve under the piriform muscle appeared without neoplastic deformations. However, the nerve showed a roughy and soft, pale constitution, with the inferior branch appearing flabby and yellowish. Since a real mass or tumor was not encountered, a complete neurolysis of the sciatic nerve was performed. A 2 cm length biopsy was taken, on the yellowish sciatic nerve branch.

On frozen section, peripheral nerve tissue with intra-neural myxoid changes were noted, but a diagnosis could not be reached. Therefore the tissue was formalin-fixed and paraffin-embedded according to routine procedures. Blocks were serially cut and sections were stained with haematoxylin-eosin, Alcian blu pH 1.00. Immunohistochemistry was performed in an automated stainer, (Ventana, Tucson, AZ, using Ventana purchased pre-diluted antibodies).

**Microscopic findings**

On histological examination (Fig. 2), the nerve appeared enlarged due to endoneural deposits of mucoid matrix, Alcian blu pH 1.00 positive, that displaced and compressed the swelled neural fibers. Epineurial tissue appeared slightly fibrotic, while perineurium and endoneurium were thickened. Inflammatory or atypical cells were not observed either in nerve

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**Fig. 1.** MRI showed a diffuse and regular enhancement along the left sciatic nerve.

**Fig. 2.** Nerve enlargement due to endoneural deposit of mucoid matrix.
fibers or in the surrounding fat tissue; surrounding blood vessels presented a minimally thickened wall. Immunohistochemistry confirmed the presence of displaced and compressed swelled axons, positive for neurofilament protein. Schwann cells, positive for S-100 protein, were rare. A perineural cellular line positive for epithelial membrane antigen, EMA was present. No floating histiocytes were detected by CD68 in the myxoid matrix. Ki-67 immunostaining did not reveal any cell proliferation. The overall histological features, together with the clinical and radiological profile were consistent with the diagnosis of "peripheral nerve mucoid degeneration".

Follow-up

After surgical neurolysis, the painful symptoms decreased, although the left leg impairment remained. At the clinical examination, eight months after surgery, the patient resulted unchanged in signs of sciatic nerve paresis, while MRI showed a slight increase in the extension of the radiological altered signal of sciatic nerve. Two years after surgery, the patient presented with an almost normal neurological picture.

Discussion

PNMD is a degenerative lesion usually appearing as a response to chronic nerve injury, caused by repeated traumatic stimulation. PNMD can be etiologically related to other reactive lesions such as traumatic neuroma, localized interdigital neuritis (or Morton neuroma), ganglion cyst, pacinian neuroma, intraneural injury neuroma. Some authors suggested a relation between PNMD, Morton neuroma and traumatic neuroma: several events following experimental nerve transaction, occurring in axons during the wallerian degeneration or other chronic trauma, are at the basis of those three entities. In addition PNMD and nerve lesion after ischemia-reperfusion injury can share similar pathogenesis. Neural damage, caused by a near joint position, similarly to what happens in intraneural ganglion cyst, has been ruled out in the genesis of PNMD. Nevertheless, the mucin deposits that characterize PNMD are most probably the result of an injury. PNMD usually involves small peripheral nerves, while major nerves, such as the sciatic nerve, are usually affected by different inflammatory or neoplastic conditions. Histologically, several reactive, inflammatory or neoplastic, lesions can show a myxoid matrix, miming PNMD. In the present case, inflammatory and reactive neoplastic lesions were excluded since PNMD usually shows no signs of inflammation or reactive proliferation changes. Benign and malignant peripheral nerve sheath tumors were easily excluded since no cellular proliferation was seen in PNMD.

In conclusion, the present case is, to the best of our knowledge, the first report of PNMD involving the sciatic nerve. Although uncommon, diagnosis of PNMD should be always kept in mind when dealing with symptomatic swelling of the major nerves.

Conflict of interest statement

None declared.

References

Large cell neuroendocrine carcinoma of the submandibular gland: a case report and literature review

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Summary
Neuroendocrine tumors (NET) are a heterogeneous group of malignancies with a broad spectrum of histomorphologies, tissue origins, and clinical outcomes, which arise from neural crest cells with neuroendocrine differentiation. Salivary gland tumors account for 3-6% of all head and neck neoplasms, while large cell neuroendocrine carcinomas (LCNEC) of the salivary gland are extremely rare, with few cases reported in literature, and only 5 cases involving submandibular gland. The rarity of these tumors in salivary glands is probably related to the scarcity of neuroendocrine cells in this tissue, whose presence is still a matter of debate. Regardless of their low frequency, it is imperative to differentiate these tumors from the much more common squamous cell carcinomas and metastatic NETs, due to different therapeutic approach and prognosis. In this paper, we report the case of a 21-year-old man, with a LCNEC involving a submandibular gland followed by several recurrences over the years. In addition, we include a comprehensive review of the available literature on this topic.

Key words
Neuroendocrine tumors • Salivary gland • Submandibular gland • LCNEC

Introduction
Neuroendocrine tumors (NET) are a group of malignant neoplasms characterized by a wide histological and clinical heterogeneity. NETs originate from the diffuse neuroendocrine system cells, and although they can occur in different organs and tissues, they appear more frequently in digestive and respiratory tracts. Primary NETs of the salivary glands are uncommon, accounting for up to 1-3% of major salivary gland malignancies, and originate almost exclusively in parotid and submandibular glands ¹. Sublingual and minor salivary gland NETs rarely occur and are difficult to distinguish from morphologically identical NETs derived from the surface mucosa of the upper aerodigestive tract. The 4th Edition of the World Health Organization (WHO) Classification of Head and Neck Tumors describes two histotypes of salivary gland NET within the group of poorly differentiated carcinomas: small neuroendocrine carcinomas (SmCC), which account for most of the salivary gland NET, and large-cell neuroendocrine carcinomas (LCNEC), which are extremely rare: indeed, the SmCC to LCNEC ratio is about 5:1 ¹ ². The rarity of NETs in the salivary glands is probably related to the scarcity of neuroendocrine cells in this tissue. Indeed, the presence and distribution of neuroendocrine cells in human salivary glands is still a matter of debate.

Case report
In August of 2005, a 21-year-old Caucasian man was referred to the Department of Maxillofacial Surgery, “Ospedali Riuniti” General Hospital, Ancona, Italy, by his general practitioner for a painful swelling in left submandibular region for 2 months. Past medical history was unremarkable. On palpation, a firm and painful small nodule was detected in that region. There were no intraoral lesions and the facial nerve was preserved. CT revealed a nodular, well-enhanced tumor,
about 2.5 cm in maximum diameter, in the left submandibular gland, with a moderate swelling of some homolateral cervical lymph nodes. The fine-needle aspiration cytology (FNAC) of the nodule was non-diagnostic. The patient underwent total left submandibular gland and lymph node removal and the material was sent to the Institute of Pathology, Marche Polytechnic University, Ancona, for histological examination. On gross examination, the submandibular gland showed a grayish-white, firm and solid nodule, measuring 2.5 x 1.8 cm. This lesion had well-defined margins, with a margin distance of 0.1 cm. On microscopic examination, the submandibular lesion was characterized by organoid growth with minimal differentiation and high mitotic rates, showing tumor invasion into muscular tissue (Fig. 1A). The tumor growth pattern consisted of sheets and trabeculae, with a tendency for coagulative necrosis (Fig. 1B). Furthermore, occasional intravascular and perivascular extension of tumor tissue was observed (Fig. 1C). The tumor was composed of large, pleomorphic (> 30 μm) and poorly differentiated neoplastic cells, with scarce eosinophilic or clear cytoplasm. The tumor cell nuclei had angulated molded shape, prominent nucleoli, arranged in solid nests, coarse and thickened chromatin with a vesicular distribution, and smudgy basophilic material surrounding intra-tumoral blood vessels (Fig. 1D). The cell borders were well-defined and occasional bizarre giant tumor cells were present. The proliferative index, evaluated with Mib1/Ki-67, was about 30-35% (Fig. 2A). The tumor cells expressed immunoreactivity for CD56, synaptophysin, AE1/AE3,
CAM 5.2, and p63 (Fig. 2B-F). No immunostaining for CK7, CK20, chromogranin A, S100, and HMB45, was observed.

Excluding some metastatic and primary tumor entities (i.e. Merkel cell carcinoma, Ewing family tumors, solid adenoid cystic carcinoma, metastatic neuroblastoma, ...
lymphomas and melanoma), based on the cell morphology, growth pattern, proliferative index, and immunophenotype, the lesion was classified as primary poorly differentiated LCNEC of the left submandibular gland.

In February 2009, the tumor recurred in the lower pole of the left parotid gland as a grayish-white capsulated nodule measuring 1.7 cm in diameter. Subsequently, in December 2015, a small nodule of the left sublingual gland was detected; it consisted of a neoplastic nodule measuring 3 x 1.5 cm with neoplastic infiltration of the left lingual nerve, morphologically similar to the previously diagnosed neoplasm. In February 2016, a third recurrence was observed on the left floor of the mouth. On gross examination, the lesion showed an ill-defined grayish-white nodule measuring 1.2 cm in the oral soft tissue, with muscle and perineural infiltration. Histological and immunohistochemical examinations confirmed the neuroendocrine nature of the tumor. In February 2018 another recurrence was diagnosed, showing multiple laterocervical white nodules up to 1 cm in diameter within fibroadipose tissues. Furthermore, diffuse lymph node involvement and distant metastases (e.g. liver, spinal column) were found.

Discussion

One of the main problems in the management of patients with NET is the lack of universally accepted standards, both for nomenclature and for disease staging. Recently, the WHO recommended a new classification system for pancreatic neuroendocrine neoplasms, while the other organs still refer to the 2010 WHO classification. The grading evaluation is performed on the basis of morphological criteria and the evaluation of the proliferation fraction according to the European Neuroendocrine Tumor Society (ENETS) scheme. This classification distinguishes differentiated NET and poorly differentiated neuroendocrine carcinoma (NEC); (a) NET G1 (mitotic counting < 2 for 10 high power fields (HPF) and/or Ki67 index < 3%); (b) NET G2 (mitotic counting 2-20 for 10 HPF and/or Ki67 index 3-20%); (c) NET G3 (mitotic counting > 20 for 10 HPF and/or > 20% Ki67 index); (d) NEC (mitotic counting > 20 for 10 HPF and/or > 20% Ki67 index). G3 NET and NEC are characterized by high mitotic and/or proliferative index values, and the only difference is the presence of well-differentiated or poorly differentiated morphology, respectively.

The clinical, histological, and immunohistochemical features of the present case are consistent with LCNEC, according to the WHO Classification of Head and Neck Tumors, and with NET G3, according to the ENETS scheme for pancreatic neuroendocrine neoplasms.

Salivary gland NETs usually affect adults with only exceedingly rare examples occurring in the pediatric population. The most common clinical presentation is a rapidly growing neck mass occurring in the parotid gland region or less commonly in the submandibular region. The diagnosis of salivary gland NET may be difficult due to the low incidence of these tumors. The diagnosis is based on histological, ultrastructural, and immunohistochemical criteria and on a comprehensive work-up to rule out the metastatic origin of the tumor. Both SmCCs and LCNECs are high-grade carcinomas characterized by organoid cellular growth with minimal differentiation, rapid mitotic activity, and frequent presence of coagulative necrosis. Palisading at the periphery of tumor nests, trabeculae, and rosettes can be encountered. The LCNEC cells have relatively abundant cytoplasm, larger nuclei with more course chromatin and prominent nucleoli.

Salivary gland LCNEC is an exceedingly rare entity: to our knowledge, there are only 15 cases reported in literature from 1990 (Tab. I). This tumor mainly affects male patients and seems to occur exclusively in adults, ranging between 21 and 88 years old (mean age 66.7 ± 23.4 years); noteworthy, this is the youngest salivary gland LCNEC reported in literature. The LCNECs usually involve the parotid gland with just 6 cases affecting the submandibular gland. The most common clinical presentation is a rapidly growing neck mass occurring in the parotid gland region or less commonly in the submandibular region. On the contrary, some patients may complain of a painful mass with facial nerve paralysis. LCNEC usually presents as a poorly defined firm mass, showing a mean size of 5.17 ± 3.29 cm, but in some cases, they are smaller, probably owing to their superficial location, which are easier to detect.

The most common diagnostic procedure used to investigate these tumors is FNAC, even if a large core needle biopsy must be preferred because small biopsy samples may be overlooked or misdiagnosed. Clinical examination and imaging techniques (ultrasounds, X-rays, CT, PET-CT, and MRI) are required to diagnose LCNEC and may help rule out the metastatic origin of the NET.

Regarding immunohistochemical findings, the most studies markers were chromogranin A and synaptophysin, showing positivity in 46.2% and 78.6% of cases, respectively. Other immunohistochemical markers were evaluated in some cases of salivary gland LCNEC, such as CD 56, CK AE1/AE3, and synaptophysin, showing positivity in all of them (Tab. I). Tumor cells may express synaptophysin, chromogranin
### Tab. I. Cases of salivary gland LCNECs reported in the literature.

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Age</th>
<th>Sex</th>
<th>Symptoms (months)</th>
<th>Instrumental investigation</th>
<th>Site (cm)</th>
<th>Gland type</th>
<th>Treatment</th>
<th>Immunohistochemical markers</th>
<th>Follow-up (months)</th>
<th>Recurrence (months)</th>
<th>Death (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hui et al. (1990)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Surgery, RxT</td>
<td>-   +</td>
<td>36</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Larsson and Donner (1999)</td>
<td>88</td>
<td>F</td>
<td>Painless swelling</td>
<td>FNAC</td>
<td>2</td>
<td>Par.</td>
<td>Surgery, RxT</td>
<td>-   +</td>
<td>5</td>
<td>Yes (4)</td>
<td>Yes (1)</td>
</tr>
<tr>
<td>Nagao et al. (2000)</td>
<td>72 M</td>
<td>Painless swelling (4)</td>
<td>FNAC, CT</td>
<td>7</td>
<td>Par.</td>
<td>Surgery, RxT, ChmT</td>
<td>-   +</td>
<td>48</td>
<td>Yes (24)</td>
<td>Yes (24)</td>
<td></td>
</tr>
<tr>
<td>Casas et al. (2005)</td>
<td>74 M</td>
<td>Painless swelling (18) facial paralysis (&lt; 1)</td>
<td>FNAC, CT</td>
<td>9</td>
<td>Par.</td>
<td>Surgery, RxT</td>
<td>+   +</td>
<td>8</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ueo et al. (2005)</td>
<td>72 M</td>
<td>Painless swelling (3)</td>
<td>CT</td>
<td>4.5</td>
<td>Par.</td>
<td>Surgery, RxT</td>
<td>+   +</td>
<td>1</td>
<td>Yes (1)</td>
<td>Yes (8)</td>
<td></td>
</tr>
<tr>
<td>Sowerby et al. (2007)</td>
<td>81 M</td>
<td>Facial paralysis</td>
<td>FNAC</td>
<td>6</td>
<td>Subm.</td>
<td>RxT, ChmT</td>
<td>-   +</td>
<td>27</td>
<td>No</td>
<td>Yes (6)</td>
<td></td>
</tr>
<tr>
<td>Chernock et al. (2011)</td>
<td>68 M</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Par.</td>
<td>-</td>
<td>+ +</td>
<td>6</td>
<td>Yes (-)</td>
<td></td>
</tr>
<tr>
<td>Petrone et al. (2013)</td>
<td>72 F</td>
<td>Painless swelling</td>
<td>FNAC, X-ray</td>
<td>1.4</td>
<td>Subm.</td>
<td>Surgery</td>
<td>+   +</td>
<td>36</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yamamoto et al. (2013)</td>
<td>58 M</td>
<td>Painless swelling (4)</td>
<td>MRI, PET</td>
<td>2</td>
<td>Subm.</td>
<td>Surgery</td>
<td>-   +</td>
<td>14</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kawaratani et al. (2013)</td>
<td>68 M</td>
<td>Painless swelling</td>
<td>FNAC, CT</td>
<td>5</td>
<td>Subm.</td>
<td>Autopsy</td>
<td>-   +</td>
<td>No</td>
<td>Death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Andreasen et al. (2016)</td>
<td>69 F</td>
<td>Rapid grow swelling (&lt;1)</td>
<td>US, FNAC, PET-CT</td>
<td>5.5</td>
<td>Subm.</td>
<td>Surgery, RxT, ChmT</td>
<td>-   +</td>
<td>19</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faisal et al. (2016)</td>
<td>45 M</td>
<td>Painfull swelling (5), facial weakness (3)</td>
<td>X-ray, FNAC, MRI, PET-CT</td>
<td>9.5</td>
<td>Par.</td>
<td>Surgery, RxT</td>
<td>-   +</td>
<td>12</td>
<td>No</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present case (2019)</td>
<td>21 M</td>
<td>Painfull swelling (2)</td>
<td>FNAC, CT, PET</td>
<td>2.5</td>
<td>Subm.</td>
<td>Surgery</td>
<td>-   +</td>
<td>125</td>
<td>Yes (41)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FNAC = Fine-Needle Aspiration Cytology; CT = Computed Tomography; MRI = Magnetic Resonance Imaging; PET = Positron Emission Tomography; Par. = Parotid; Subm. = Submandibular; RxT = Radiotherapy; ChmT = Chemotherapy; Chr = Chromogranin; Syn = Synaptophysin.
A and/or CD56; indeed, the NETs stain for at least one of these known neuroendocrine markers. Electron microscopy could facilitate the diagnosis showing some neuroendocrine features such as 100-275 nm sized dense-core granules within the cytoplasm of tumor cells. Immunohistochemical staining is helpful to distinguish other malignant large cell neoplasms, such as melanoma and Merkel cell carcinoma arising in parotid or submandibular regions. Surgical resection of the affected gland is the treatment of choice; however, in two cases it was not performed. Three patients received only surgical resection, while radiotherapy and chemotherapy were associated in 8 and 3 cases, respectively. Only in two cases a combination of surgery, radiotherapy, and chemotherapy was used. The mean follow-up time was 28.1 ± 32.2 months and recurrences were reported in 5 patients, showing a disease-free survival time of 17.5 ± 11.8 months. Currently, there are no guidelines to treat salivary gland NETs and the main therapeutic strategies generally derive from other tumor types, such as cutaneous Merkel cell carcinoma. Surgery is the gold standard for well differentiated NETs with most patients undergoing local resection and neck dissection, even though there is a lack of agreement about the need of elective neck dissection in these cases. The majority of patients receive adjuvant radiotherapy, although its effectiveness has not been demonstrated. Chemotherapy seems to be ineffective as well, although the responsiveness of these lesions to different types of chemotherapeutic drugs has not been well documented.

In conclusion, LCNEC of the salivary glands represents a diagnostic and therapeutic challenge due to its extreme rarity. The definitive diagnosis is based on histological evaluation, demonstrating the presence of typical neuroendocrine architecture with positivity to neuroendocrine markers. Imaging is necessary to exclude metastatic origin of the tumor, since NETs occur more frequently in other body regions, or to differentiate these tumors from the much more common squamous cell carcinomas, because the therapeutic approach and prognosis are significantly different. For localized tumors, surgery seems to be the first therapeutic option, supplemented by radiotherapy and/or chemotherapy, but studies with larger sample size are advised to establish guidelines.

Conflict of interest statement
None declared.

References


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Case Report

Russell body gastritis

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Summary

Russell body gastritis is caused by an accumulation of plasma cells within the gastric mucosa. These plasma cells are characterized by eosinophilic cytoplasmic inclusions of immunoglobulin which are called “Russell bodies.” We report a case of Russell body gastritis in a 28-year-old male who presented with abdominal pain and rectal bleeding. Endoscopy showed erosions with edema and vascular congestion in the gastric body and antrum. The biopsy showed chronic gastritis with plasma cell infiltration of the lamina propria. Many plasma cells contained cytoplasmic Russell bodies which stained positive for CD138, CD79a, Kappa and lambda light chains. The Russell bodies were negative for pancytokeratin, excluding signet ring cell carcinoma. Russell body gastritis is an uncommon, benign reactive condition.

Key words

Gastric biopsy • Gastritis • Plasma cells • Russell body gastritis

Russell body gastritis is characterized by the presence of plasma cells with eosinophilic cytoplasmic inclusions of immunoglobulin in the gastric lamina propria. The process is thought to be a benign reactive process. Initial case reports suggest the association of this entity with infections, particularly Helicobacter pylori infections.¹² We report a case of a 28-year-old male with a history of human immunodeficiency virus (HIV) who presented with abdominal pain, fatigue and a one week history of rectal bleeding. Complete blood count showed pancytopenia. Computerized tomography (CT) scan showed moderate splenomegaly and mild hepatomegaly. Endoscopy revealed erosions, erythematous mucosa, and vascular congestion in the gastric body and antrum. Microscopic examination showed an accumulation of plasma cells with cytoplasmic Russell bodies in the lamina propria. The plasma cells had eosinophilic cytoplasm and were present in a background of chronic inactive gastritis (Fig. 1). Immunohistochemical stains were positive for CD138, CD79a and they show polyclonal expression of kappa and lambda light chains (Fig. 2). The Russell bodies were negative for pancytokeratin, excluding a signet ring cell carcinoma. Giemsa stain is negative for H. pylori organisms. There are occasional case reports of Russell body gastritis in the literature¹⁻¹⁴ and some describe an association with H. pylori infection.²⁻⁵ Theories regarding the cause include chronic gastritis leading to either under-secretion of immunoglobulin by plasma cells or over-production of plasma cells with increased formation of Russell bodies.⁵⁻⁶ Some cases have been described in H. pylori negative patients⁹⁻¹³, and occasional cases have been reported in HIV positive patients⁴⁻⁷⁻¹⁰. Shinozaki et al. reported two cases of Epstein-Barr virus positive stomach carcinoma with associated accumulation of Mott cells.⁶ Altindag SD et al. studied 11 cases of Russell body gastritis and observed one case of concomitant carcinoma and plasma cell neoplasm.¹⁴ The presenting symptoms were variable and mostly non-specific.¹⁴ Endoscopic examination can show features of gastritis and erythematous mucosal changes or present as nodular or raised lesion.¹²⁻¹⁵. Follow-up is suggested due to occasional reported cases with associated concurrent neoplastic processes.¹⁴⁻¹⁵. The differential diagnosis includes plasma cell neoplasms, other hematologic neoplasms, histiocytic pro-
**Fig. 1.** Gastric mucosa with chronic inflammation and presence of plasma cells with intracytoplasmic eosinophilic globules. (A): 100X, (B & C): 200X and (D): Pancytokeratin immunohistochemical stain which is negative in the globules (400x).

**Fig. 2.** These eosinophilic globules are positive for CD79a and CD138 (A & B, 400X, respectively). Lambda and kappa immunohistochemical stains are also positive (C & D, 200X, respectively).
cesses, and signet ring cell adenocarcinoma. Immunohistochemical stains are helpful in excluding the above entities as diagnostic possibilities. Wolkersdörfer et al. described concomitant presence of monoclonal gammopathy of undetermined significance, H. pylori infection and Russell body gastritis. Plasma cell neoplasms are monoclonal and they express either kappa or lambda light chains. Our case shows expression of both kappa and lambda light chains which is an expected finding in Russell body gastritis unassociated with a plasma cell neoplasm. There have been case reports of Russell body gastritis with monoclonal expression of kappa or lambda light chain. Histiocytic infiltrates may have a similar histologic appearance with eosinophilic cytoplasm and immunohistochemical stains for CD68 and CD138, a plasma cell marker can aid in the differential diagnosis. Signet ring cell adenocarcinoma may have a similar appearance, with the malignant cells often having nuclear atypia and cytoplasmic mucin secretion. Immunohistochemical stains for keratin are positive in signet ring cell adenocarcinoma and negative in Russell body gastritis. We report a case of Russell body gastritis in an H. pylori negative patient who is HIV positive and on HAART (highly active antiretroviral therapy) therapy. Russell body gastritis is considered to be a reactive process.

**Conflict of interest statement**

None declared.

**References**


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In the conviction that a look at the past can contribute to a better understanding of the present in the field of science too, we discuss here two aspects of the relationship between early 20th century anatomic pathology and psychiatry that have received very little attention, in Italy at least. There was much debate between these two disciplines throughout the 19th century, which began to lose momentum in the early years of the 20th, with the arrival on the scene of schizophrenia (a disease histologically sine materia) in all its epidemiological relevance. The First World War also contributed to the separation between psychiatry and pathology, which unfolded in the fruitless attempts to identify a histopathological justification for the psychological trauma known as shell shock. This condition was defined at the time as a “strange disorder” with very spectacular symptoms (memory loss, trembling, hallucinations, blindness with no apparent organic cause, dysesthesias, myoclonus, bizarre postures, hemiplegia, and more), that may have found neuropathological grounds only some hundred years later. Among the doctors with a passed involvement in the conflict, Ugo Cerletti, the inventor of electroshock treatment, focused on the problem of schizophrenia without abandoning his efforts to identify its organic factors: if inducing a controlled electric shock, just like an experimentally-induced epileptic seizure, seems to allay the psychotic symptoms and heal the patient, then what happens inside the brain? In seeking histological proof of the clinical effects of electroconvulsive therapy (“the destruction of the pathological synapses”), and attempting to isolate molecules (that he called acroagonins) he believed to be synthesized by neurons exposed to strong electric stimulation, Cerletti extended a hand towards anatomic pathology, and took the first steps towards a neurochemical perspective. However his dedication to finding a microscopic explanation for schizophrenia – in the name of a “somatist” approach that, some years earlier, the psychiatrist Enrico Morselli had labelled “histomania” – was unable to prevent psychiatry from moving further and further away from anatomic pathology.
microscopical descriptions. Besides, he classified other mental diseases characterized by dementia with a well known histopathological basis in neurosyphilis, trypanosomiasis (sleeping sickness), senile dementia and atherosclerotic dementia. After clearing the field of known conditions and diseases of other kinds, Emil Kraepelin had little faith in an anatomo-pathological classification of psychoses, despite postulating their organic basis. Then, in the first decades of the 20th century, the huge issue of schizophrenia came to light, a nameless ghost for the pathologist. This brought the curtain down on the conviction that “mental diseases are diseases of the brain”, as Wilhelm Griesinger (a clinician and neuropathologist very influential in the second half of the 19th century) had put it. In the eyes of the psychiatrist, it also marked the end of that special status of pathology in medicine that Foucault had described as “the privileges accorded to pathological anatomy.”

The outbreak of the First World War brought psychiatrists face-to-face with hitherto unknown situations and mindscapes, once again without histopathologically based solutions. The effects of mental traumatisms in wartime (shell shock) and the reports on treatment with electroconvulsive therapy (so called faradism), let a track in the background of doctors involved in the conflict as Ugo Cerletti and others.

Shell shock: a late rapprochement that came too late

As an extreme experiment on how the human mind adapts to traumatic phenomena, the war provided a tragic opportunity to test opposing theories on the pathogenesis of the soldiers’ psychiatric disorders. Once the insinuation that soldiers were largely simulating their symptoms had been rejected – with some difficulty, and never completely by the world of military medicine – two different opinions emerged. According to some, war does not make people ill, it only brings out latent psychological impairments. This was the view taken by numerous physicians in countries all over Europe, and in Germany by Alois Alzheimer, who lived only into the first few months of the Great War (he died in 1915). This view was also supported by the majority of Italian psychiatrists, who had inherited Lombroso’s ideas. According to others, people unavoidably become ill in war, as the experimental psychologist Agostino Gemelli saw on the front line in 1917. He wrote of the impoverishment of the inner life of the soldiers (what he described as the “shrinking field of consciousness of the infantryman”) 11, who were useful only as unthinking launchers of an assault. It was in the British scientific publications of the time, in 1915, that shell shock first became a hot topic. Then Freud’s studies on traumatism in wartime, what he called traumatic neurosis as part of his drive theory, and other studies presented at a conference of psychiatrists amply dedicated to the psychological trauma of war in Budapest in 1918 15, anticipated modern historiography in consolidating this psychopathological interpretation of man in wartime, or in other words of war as a pathogenic agent.

The psychological disorders of the traumatized infantryman (3 to 5% in the British army) could produce all sorts of symptoms: asthenia; amnesia; headache; vertigo; insomnia; hallucinations; nervous tics; aphasia; stammering; deafness and blindness with no apparent organic cause; tachycardia; arrhythmias; trembling; myoclonus; spastic muscle contractions or their opposite, flaccid paralysis, even to the point of hemiplegia; lack of appetite; sphincter disorders; and cutaneous paresthesias, anesthesias and hyperesthesias. By the end of the war, even many of the psychiatrists who had originally taken Lombroso’s approach had come to admit that wartime trauma can cause a diencephalic-mesencephalic neurovegetative lesion, with effects on the cranial nerves and systemic repercussions, though they would hasten to say that this could only happen to predisposed individuals, who they described as “constitutionally cestopathic”.

Could we claim that this also paved the way to anatomico-pathological and in particular neuropathological investigations? So it seems, although autopsies were certainly not routine practice at the front. In Italy, for

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4 In Italy, where a strong impression was left by Camillo Golgi, there was a robust histopathological tradition that opposed the clinical taxonomic approach to psychological disorders. But even in Germany psychiatry did not develop in a linear manner, and Kraepelin’s strict clinical prognostic classification stood in opposition to the firmly organicist approach of Karl Wernicke.

5 If autopsies had been conducted more often at army hospitals it might have been possible to discover the myelotoxic effects of mustard gas almost 30 years sooner. Its effects were accidentally acknowledged only during the Second World War: Allied bombing on the port of Bari in 1943 released mustard gas from the hold of a ship that was hit, and dissections conducted on the civilians who died a few days later revealed total bone marrow aplasia. These observations also led to the first controlled studies on chemotherapy for acute leukemia. It is also thanks to such studies that we know about the neuropathological effects of punctate hemorrhages.
instance, dissections were being conducted for teaching purposes at the army university in San Giorgio di Nogaro, near Palmanova, behind the front lines in the north-eastern Veneto region, until this extraordinary medical school experiment was interrupted by the crushing defeat suffered at the Battle of Caporetto. In his book L’Officina della Guerra, Antonio Gibelli dedicates more than one chapter to the topic of the traumatized infantrymen who “not even the most ferocious discipline succeeded in controlling”, concluding that specialists on every front would be wondering for years about the pathogenesis of this “strange disease” without succeeding in finding an answer. It is a state of concussion that grips a soldier who feels a cannonball whizz by, that vent du projectile known ever since the time of the Napoleonic wars. But what could the pathologists see in the brain of the handful cases of dead soldiers they examined who had not been exposed to gas, physical injury or direct trauma, but who had the symptoms of shell shock? Frederick Walker Mott, the pathologist who studied the problem more than any other at the time, spoke of congestion of the meningeal and intraparenchymal vessels, and initial signs of chromatolysis of the nuclei in the motor areas of the frontal gyri, pons, and medulla oblongata. These findings are rather vague and scarcely convincing, bearing in mind the delay in the fixation of the brain tissues attributable to the unavoidable logistic limitations of autopsies conducted in wartime circumstances, and the different fixing agents used (Kaiserling solution, alcohol). There was also evidence of sparse, tiny hemorrhagic petechiae in the white matter of the centrum semiovale, corpus callosum, internal capsule and subarachnoid spaces, in the absence of any external signs of trauma. The pathologist concluded that: “undoubtedly the vast majority of non-fatal cases of shell shock are more emotional in origin than commotional, and occur especially in subjects with an inborn neurotic or neuropathic temperament”. In another study, the same author hypothesized that fatal cases had involved damage to the extracellular matrix, “the delicate colloidal structures (...) arresting the function of the vital centers in the medulla”. About the existence of predisposed individuals, he wrote that “the moral effect of the continuous anxious tension of what may happen [under artillery bombardment], which, combined with the terror caused by the horrible sights of death and destruction around, tends to exhaust and eventually even shatter the strongest nervous system”. In short, we could say that – from a histopathological standpoint – the genesis of shell shock remains unknown. The review conducted by Peter Leese, Hans Binneveld and Ben Shepard on a large number of articles and monographies about shell shock published in England between 1915 and 1920 confirmed that efforts to find etiological explanations of this condition came to a dead end.

Traumatic shock experienced in times of war was classified as a clinical disorder with the introduction in the DSM-III for diagnosis of post-traumatic stress disorder (PTSD). This condition is characterized by intense fear, reactualization of the traumatic episode, avoidance of stimuli associated with the trauma, and increased arousal. Modern research approaches have found evidence of neurological changes associated with some types of trauma (including wartime trauma, but also sexual abuse by family members). For instance, imaging methods documented changes in the volume of the right hippocampus (limbic system) in Vietnam war veterans, and other alterations in the brain. These changes are similar in some ways to those identifiable in animals submitted to prolonged stress, which are accompanied by high cortisol levels.

Although there are still many aspects to clarify, the modern conception of PTSDs essentially focuses on the involvement of procedural memory (or implicit memory), while explicit recall may even be completely lacking. In other words, patients suffer from anomalous memorization processes that tend not to regress spontaneously. These memories may be fragmented and inaccessible, or only partially accessible, for conscious recall. The condition is therefore characterized by a distortion of the meaning of perceived reality and individual subjectivity due to the effects of tumultuous emotions, and by fragments of intrusive, painful memories that are difficult to manage. It is only recently, moreover, that the first histopathological data have emerged to support an organic basis for the symptoms of traumatism, the so-called

caused by blister gases (yperite) or other suffocating toxic gases used in war (mixtures of chlorine and phosgene on the Italian front line) that pathologists learned to identify already during the First World War, and judged responsible for arteriolar thrombosis. But the most significant increase in the amount of autoptic activity, on the Western front at least, only came in the final months of 1918, coinciding with the outbreak of the Spanish flu epidemic. "I’m afraid of going crazy", he told me. "I’m going to go crazy one of these days, or I’m going to kill myself. I’ve got to kill myself." I didn’t know what to say. I, too, could feel the ebb and flow of waves of madness. At times I could feel my brain sloshing around inside my skull, like water inside a shaken bottle. (from A Soldier on the Southern Front, by E. Lussu). For a more complete picture of the phenomenon it is worth taking a look at the other side of the front too, and Ernst Junger’s touching descriptions of the soldiers’ condition. For a review on the topic, see also ref. 8.
chronic-blast traumatic brain injury (TBI). Here again, only a limited number of cases have been analyzed, on the brains of soldiers returning from military campaigns in Iraq with PTSD (and suffering from headaches, anxiety, insomnia, memory loss, depression, epileptic seizures, and chronic pain) who subsequently died of other causes, including substance abuse or suicide. The common denominator of their TBI seems to be astroglial fibrosis, revealed by immunohistochemical staining for GFAP. This involved an increase in fibrosis at the interface between the white and grey matter, in tissue adjacent to the cerebrospinal fluid, around the penetrating arteries, around the basal nuclei and limbic system — in other words, at the interface between areas of different physical density invested by the gaseous wave of the explosion. The damage can explain the symptoms: headache due to tissue disruption of pia and injury to penetrating vessels, with an altered circulation of the CSF; cognitive impairments caused by damage to the “U” fibers at the interface between the grey and white matter; and memory deficits and sleep disorders due to damage to the periventricular structures of the limbic system. It is interesting that the same types of lesion were found in the brains of soldiers and victims of acute-blast TBI too, supporting the hypothesis of an early onset of this fibrotic damage (which is not seen in controls exposed to trauma not caused by explosives, as in cases of chronic traumatism, or trauma caused by contact sports or road accidents). It could be said emphatically that, a hundred years on, neurophysiology and modern pathology provide us with a new hypothesis to explain shell shock, very different from the moralistic explanations (cowardly soldiers), Lombroso’s theories (genetic shortcomings in some soldiers), or purely psychoanalytical interpretations of the past.

Electroshock between psychiatry and pathology

In the early 20th century world of psychiatry, there were still those who were striving for a quick fix for certain psychiatric disorders, with the aid of hypnosis, for instance. During the First World War, there were even more evident signs of this drive to find rapid and effective therapies that would enable soldiers to be promptly returned to the front line, relying on the institution of the so-called psychiatrie de l’avant (prompt intervention behind the front line), and the provision of intensive treatments in city hospitals. The records of the London National Hospital report on shell shock being treated with electroconvulsive therapy (called Faradism) (Fig. 1) combined with massage, baths, heat, exercise, and suggestion (hypnosis). In actual fact, as the historian of medicine Giorgio Cosmacini reports (personal communication), already in the second half of the 19th century increasing use was being made in hospitals of electrotherapies that involved administering a shock or “sharp jerk” to patients with motor disorders and various other kinds of impairments.

It is against this background that electroconvulsive therapy (ECT) was invented by an eclectic clinician (Ugo Cerletti) who fought in the First World War and was consequently certainly able to observe the effects of the vent du projectile on the soldiers. However, Cerletti’s interest focused mainly on finding a treatment for the disease of the century, schizophrenia. At the time, it was common to treat this condition using physical means (hydrotherapy, light baths, sedatives), unless the clinician opted for a frontal lobotomy. Without arriving at such an extreme solution, severe cases were treated with insulin- and acetylcholine-induced shocks and, from 1936 onwards (with results that seemed very encouraging at the time), with the cardiazol-induced shock introduced by Lazlo von Meduna, a Hungarian scientist of international standing in close contact with Cerletti.

Cerletti (Fig. 2) trained in Germany as an anatomic pathologist, and held a strong belief in the concept of “somatism”. For years, he studied epilepsy and its

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As Valeria Babini wrote, Freud introduced the topic of repetition compulsion, and consequently of the death drive, starting from a reflection on traumatic neurosis.
neuroanatomical grounds, accumulating a considerable amount of experience in research and on the wards (at the Mombello psychiatric hospital in Milan, at the Universities of Bari and Genova, and finally at the Sapienza University in Rome)\(^6\). His investigations began with histological studies on the brains of animals exposed to cardiozol-induced shock. Cardiozol causes an epileptic seizure, and epilepsy was a model of great interest to psychiatrists at the time. They saw a clinical, somatic (of athletic type in schizophrenia, leptosomic in epilepsy), and statistical incompatibility between epilepsy and schizophrenia. According to von Meduna, this incompatibility applied to the pathological sphere too: after analyzing histological preparations of brain tissue, he wrote about the contrast between the excessive growth of glia cells in epilepsy and “the apparent torpor of the glia system in the schizophrenic brains”\(^42\). By analogy, Cerletti studied the effect of electroshock on animals\(^43\), finding it capable of producing a controlled or “fractionated” epileptic seizure of variable intensity, that the Italian scholar observed for the first time at the Testaccio slaughterhouse in Rome. He subsequently reproduced the phenomenon in animals of various species, from Komodo dragons to penguins, from porcupines to boa constrictors, which were made available by the zoo in Rome\(^41\)\(^44\).

These experimental studies, also published in *Pathologica* in 1934\(^45\), continued after the introduction of ECT in clinical practice in 1938. Its clinical efficacy was so much greater than that of any other previously-attempted therapies that the diffusion of this treatment was immediate, global and destined to have a fundamental role in psychiatric treatments for more than 20 years\(^5\). Studies on the brains of treated animals were ambitiously aimed to discover the organic basis for mental disorders by starting from the effects of the therapy proving the most effective in humans\(^46\). They revealed the onset of “glial pyknosis, regressive vascular modifications, and pyknosis of the Purkinje cells”, though Cerletti judged these last alterations to be partly due to artefacts. At a voltage sufficient to extinguish the most severe psychiatric symptoms, the findings in mammals, pigs, and dogs became more hazy and diffuse, and Cerletti wrote that “these changes seem reversible”\(^44\). This did not prevent him from hypothesizing (albeit without succeeding in documenting it clearly) the destruction of “pathological synapses” and damage to associative pathways implicated in the genesis of schizophrenia\(^44\). Cerletti believed that these pathways developed after the brain’s ontogenesis, and were consequently more vulnerable to the insult caused by the electroshock.

Such conclusions may seem naive nowadays, in the light of modern concepts of neuroplasticity and our understanding of how the brain’s structure and functions are constantly being remodeled. However it has to be said that, though he was working in a scientific world before the most important ultrastructural, biochemical, neuroendocrinological, pharmacological and genetic discoveries, Cerletti was already attenuating what he called “histological tautologies”, right from his early studies. He became convinced that “the fundamental morbid core of the schizophrenic psyche”\(^43\) lay deeper down, in the meso-diencephalic regions, and that it was strictly linked to phenomena of a biochemical, quantifiable and identifiable nature\(^44\)\(^1\). Cerletti never abandoned his search for the organic roots of schizophrenia, based on a “somatist” approach in

\(^{1}\) For an interesting review of neuropathological studies on schizophrenia, pooling both morphological and molecular data, see two reviews by P.J. Harrison et al.\(^46\)\(^47\). The same authors recommend caution in considering their interpretation, but certainly the claim\(^46\) that “schizophrenia is the graveyard of neuropathologists” seems less pertinent today.
which he firmly believed. He consequently applied himself to attempting to isolate substances that could be synthesized by the brain during the course of ECT (“vitalizing substances of extreme defense”) 41 44. He prepared emulsions of brain tissue from treated animals, named these substances acroagonins, and administered them to patients. Though these attempts were destined to lead nowhere, they mark a change in the course charted by neuropsychology, which moved more towards the study of neuromediators. Cerletti thus realized the need to go beyond his own invention, to assure patients the benefits of ECT without the side-effects that it carried at the time (and no longer carries today, in the patients with severe drug-resistant psychiatric disorders in whom it is still used) 49. Cerletti’s name also felt the burden of these investigations 41, particularly in the 1960s and 1970s, for inventing a treatment that had made him infamous or, at best, exposed him to a degree of damnatio memoriae 40.

Conclusions

“There was a time when certain psychiatrists would not have been considered proper scientists if they had not focused their best energies as researchers on the mortuary slab, the microscope, and laboratory work” 50, wrote Enrico Morselli, a fundamental figure in the history of Italian psychiatry. He was one of the most influential clinicians in the early 20th century and it was he who labelled histological studies on mental disorders as “histomania”. It was true that, all too often, autopsy left psychiatrists dumbfounded 51. Even the studies on the psychological trauma induced by explosions (an unprecedented opportunity for investigating the relationship between symptoms and supposed lesions), and the research done by Ugo Cerletti on the effects of electroshock contributed to the downfall, in the early decades of the 20th century, of a certain idea of histological malleability of the brain. Psychiatry went in other directions, albeit with some delay in Italy attributable to a diffidence blanketed in “positivism” regarding psychoanalysis, and to the advent of the Fascist autarky in the sphere of science. The path taken by psychiatry was dictated by the knowledge available at the time, which suffered from the absence of the modern neuroscience, and particularly the advances made by molecular biology and psychopharmacology, but the discipline was already oriented towards occupying its own space in the scientific world, and not biology or abstract science of the spirit. Nevertheless, the drive towards “dissecting” the psyche, and the belief in the feasibility of breaking it down into simpler elements under the effect of morphine, sleep or hypnosis, was born in minds of Freud and Charcot, also because of the anatomic and histopathological imprint on their scientific education.

Conflict of interest statement

None declared.

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Errata

Hemangioma of the umbilical cord with associated amnionic inclusion cyst: two uncommon entities occurring simultaneously

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