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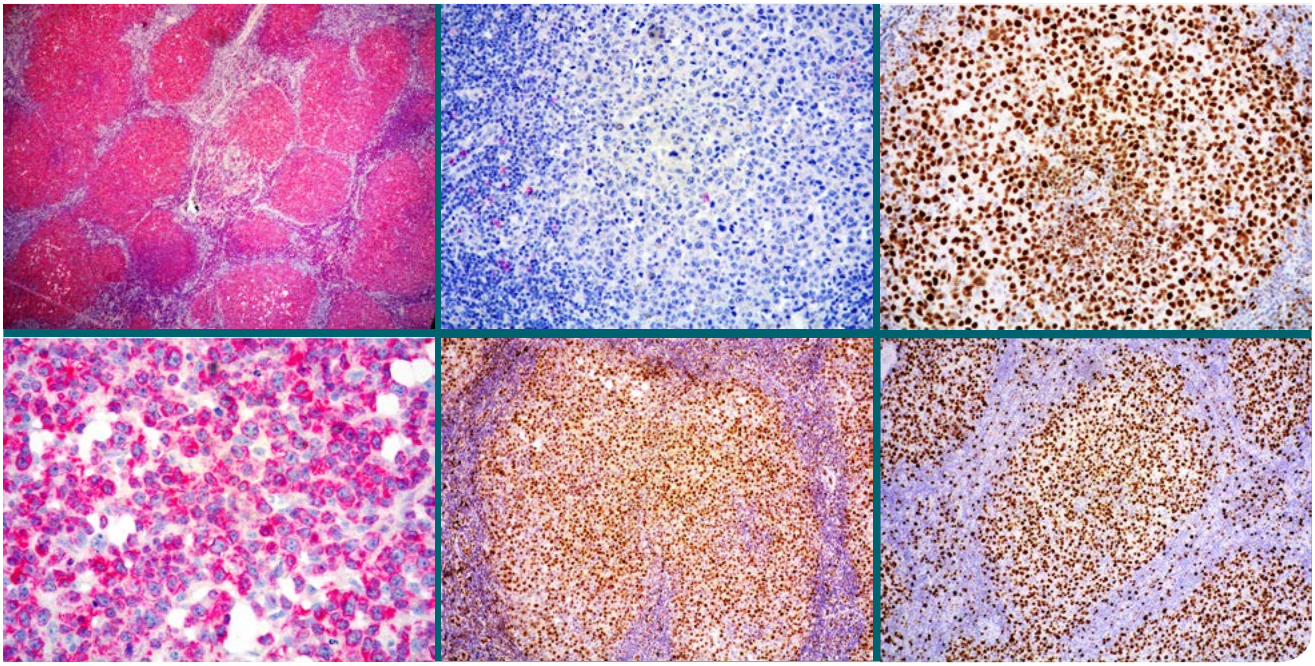

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Società Italiana di Anatomia Patologica e Citopatologia Diagnostica,
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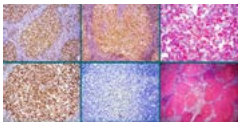

Front cover: A Lymph node. Follicular lymphoma 3B. CD20 immunostaining, 40x; B Lymph node. Follicular lymphoma 3B. CD10 immunostaining, 200x; C Lymph node. Follicular lymphoma 3B. BCL6 immunostaining, 200x; D Lymph node. Follicular lymphoma 3B. BCL2 immunostaining, 400x; E Lymph node. Follicular lymphoma 3B. LMO2 immunostaining, 100x; F Lymph node. Follicular lymphoma 3B. KI-67/MIB1 immunostaining, 100x. (page 86).

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Editorial

Covid-19 current advice for pathologists

John Firth

Consultant Histopathologist, Royal Free Hospital, Pond Street London; UK Member of the European Union of Medical Specialist (UEMS) Pathology Board, Head of UK Delegation to UEMS

Covid-19 current advice for pathologists

The emergence of a new variant of Coronavirus from its first appearance in Wuhan province, China in December 2019 [1] has become pandemic as judged by the World Health Organisation [2]. The virus has been named Severe Acute Respiratory Syndrome Coronavirus 2 and the disease Covid-19 [3]. There is no current vaccine and the best means of preventing the illness is avoiding contact with the infected person [4]. Whilst the overall mortality rate is significantly below previous similar outbreaks of coronavirus such as the Middle Eastern Respiratory Virus (MERS) and Severe Adult Respiratory Syndrome-associated coronavirus (SARS-CoV) [5]. there is a higher risk of mortality in the elderly and those with comorbidities [6]. As well as guidance on patient management [7] the World Health Organisation (WHO) gives a high priority to the protection of healthcare workers and there has been a large number of documents published by WHO which is freely available [9 10]. as well as from the European Centre for Disease Control [11]. It has long been recognised and planned for in the UK that Pandemic Flu may lead to an excess death rate over a 15 week period. For the current pandemic the mortality rate is in keeping with the estimate of 2.5% and these extra deaths will put an increased burden on public and hospital mortuaries [12].

Deaths in hospitals from pandemic flu is likely to be assessed as a natural cause of death though some EU member states have given out their own statement regarding Covid-19 Deaths with respect to referral to the Coroner or Medical Examiner, as a notifiable disease and the need for a medico legal autopsy. This will vary from state to state [13]. It is inevitable that autopsies will be performed on patients succumbing to Covid-19 and mortuary staff and pathologists will be exposed to potentially infective material from cadavers. Whilst it is still unclear the degree that one can be infected through touching inanimate objects it has been shown that the virus can persist for up to 4-5 days on a variety of surfaces [14].

As well as the UK government (HMG) giving general infection and prevention advice reference is also given to the handling of dead bodies in its most recent publication [15]. In addition the HMG has published a document regarding investigation and initial clinical management [16]. However neither document gives significant guidance on autopsies.

The Royal College of Pathologists of the United Kingdom has produced a very valuable briefing regarding autopsies on cases or suspected cases of Covid-19 [17]. The document is very useful in giving the most up to date information for the Pathologists and Mortuary technician but also begs the question of whether an autopsy should be done beyond taking the appropriate swabs to exclude or confirm Covid-19. Particularly for

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deaths in the community brought into the mortuary or sudden deaths in the emergency department, where the length in hospital may be short and the clinical history is vague, consideration should be made to perform nasal and oral swabs post mortem and await the results. Only after the results of these swabs should the pathologist then perform the autopsy, if necessary after consultation with the Coroner or their equivalent. This process should be strongly considered [17]. A further article is due to be published in the next two weeks which will consolidate this [18].

At the time of writing this Pandemic Flu has already had a devastating effect globally both at a society level and an economic level. This pandemic may well exceed the impact of the 1918 flu pandemic [19]. However the expertise and devotion of healthcare workers will eventually restore order and balance, though probably the world will have changed in some way as it has from previous pandemics.

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Editorial

What are the priorities of pathologists' activities during COVID-19 emergency?

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COVID-19 has urged all sanitary staff in a common effort of solidarity. In Italy, doctors and nurses of all ages, even those not yet entered in or already retired from the National Health System, have answered to the call from hospitals to give their support to face COVID-19 emergency.
The Pathologists community is involved in this crisis as well; thus, we have to consider what our priority activities are in this moment of a dramatic health emergency. First of all, our main duty is to maintain a high level and an optimal turnaround time of routine diagnostic activity. Cancer, as well as other serious diseases, continues to exist and need pathological diagnoses; transplants continue to request our immediate diagnostic support. Technicians, biologists and medical doctors of pathology units have to manage the correct workflow of samples to provide diagnoses in due time. Unfortunately, in our Country pathology staff have begun to be infected as well. For this reason, we must address crucial technical and organizational aspects to contain the biological risk, preserving as much as possible the quality of tissue/cell samples and the health of staff.
The Italian Society of Surgical Pathology and Cytology (Società Italiana di Anatomia Patologica e Citologia - SIAPEC) produced a document on biosafety in surgical pathology in the era of SARS-Cov2 pandemia, published in our journal *Pathologica* [1]. In this document we pointed out that all fresh/inadequately fixed specimens could be potentially infected and we addressed this problem by drawing up specific recommendations. For example, Italian pathologists have been among the first to introduce the under-vacuum and cooling technology for optimal tissue preservation of surgical samples in a formalin-free environment. This technology has the advantage of an adequate control of the so called "cold ischemic" period, but implies that almost all surgical specimens are received fresh in the laboratory and are manipulated and sampled prior to fixation. Given the actual pandemic situation, the biosafety document suggests to suspend this kind of approach and return to conventional formalin fixation, if high-level biosafety conditions cannot be completely assured. The document focuses also on intra-operative diagnoses on frozen samples and on unfixed/inadequately fixed cytological samples (e.g. effusions, bronco-alveolar lavages, etc). It suggests protocols aimed at reducing infectivity risks, while maintaining adequate conservation of morphological and biological features.
Another important concern are autopsies. SIAPEC together with the Scientific Society of Hospital Forensic Medicine of the National Health

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System (COMLAS) produced a document on the management of the corpse with suspect, probable or confirmed COVID-19 respiratory disease [2]. The document specifies each step and all procedures needed to perform autopsies in safety conditions. The criteria for the definition of cases as COVID-19 suspect, probable or confirmed are the same as those used to evaluate the possible risk of infection in living patients. Our document is in keeping with the CDC [3] and WHO [4] criteria, starting from the need of "Airborne Infection Isolation Rooms (AIIRs)" where to perform the autopsies to the appropriate use of personal protection equipment. In the document we highlight that a diagnosis of COVID-19 is based on the detection of the virus on nasal and oropharyngeal swabs with PCR technique, an appropriate clinical picture and on Computerized Tomography (CT) findings of lungs. Therefore, an autopsy with histological examination of tissue samples does not have a primary diagnostic role. If the collection of tissue samples is considered essential for the diagnosis, we propose to use percutaneous core biopsy sampling, albeit with the limits of such procedures performed on corpses.

Post-mortem histopathological findings could play a role in understanding the pathophysiology of the SARS-CoV-2 infection. For this reason, our effort should be to gather the gross and histological findings of autopsies performed in different pathology units around the country to rapidly produce a tissue and data collection useful to define the main causes of death and the various microscopic alterations induced by SARS-CoV-2.

For autoptic activities, due to the current spread of the disease and the reported false negative rate of naso-pharyngeal swabs, it is mandatory to consider all corpses as potentially infectious. Therefore, all autopsies should be restricted to well-motivated cases and performed in accordance with strict biosafety rules.

In conclusion, we believe that routine diagnostic activities of surgical pathology are a priority, because patients need our diagnoses. At the same time, it is our obligation to safeguard the health of pathology staff by strictly following all biosafety procedures.

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Guidelines

Biosafety in surgical pathology in the era of SARS-Cov2 pandemia. A statement of the Italian Society of Surgical Pathology and Cytology

Mattia Barbareschi[1], Valeria Ascoli[2], Emanuela Bonoldi[3], Alberto Cavazza[4], Romano Colombari[5], Ilaria Cozzi[2], Emanuele Dainese[6], Fabio Facchetti[7], Guido Fadda[8], Gerardo Ferrara[9], Filippo Fraggetta[10], Paolo Graziano[11], Giancarlo Murer[12], Esther Diana Rossi[8], Giulio Rossi[13], Giovanni Negri[14], Gianfranco Zannoni[8], Anna Sapino[15,16]

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Introduction

Surgical pathology units face chemical and biological risks. While chemical risks have been intensely evaluated since the formalin ban, less attention has been drown to biological risks. The actual epidemiologic situation due to the SARS-CoV-2 pandemia has raised a series of questions, which need to be addressed as soon as possible. We have to pursue two lines of action: on one hand we must immediately adopt urgent measures to reduce the risk of SARS-CoV-2 infection of laboratory personnel, and on the other hand, we must address crucial technical and organizational aspects of biological risk reduction, preserving as much as possible the quality of tissue and cell samples.

The evaluation of biological risk is an analytical process which involves different steps: a) characterization of the hazard (also known as risk assessment) and b) definition of a risk reduction strategy (also known as risk mitigation) [1].

Risk assessment implies a) the identification of the intrinsic biologic characteristics of the infectious agent, and b) the identification of the laboratory procedures related to the agent.

The intrinsic biologic characteristics of infectious agents are classified in 4 risk groups (RG) by the laboratory biosafety manual of the WHO [2]. The RG range from level 1 (RG1) which includes microorganisms that are unlikely to cause human or animal disease, to level 4 (RG4) which includes pathogens which cause serious diseases, that can be readily transmitted from one individual to another, and for which effective treat-

ment and preventive measures are not usually available.
Risk mitigation includes the definition of the appropriate a) level of biosafety of the laboratory, b) type of personal protection equipment (PPE), c) type of infrastructure and equipment, and d) education of involved personnel.
Laboratory biosafety is graded in 4 levels (BSL-1 to BSL-4) as exhaustively described in the laboratory biosafety manual of the WHO [2], and these levels are usually also defined by law (in Italy by the D. Lgs. 81/2008). BSL are a series of protections, which include individual safeguards designed to protect laboratory personnel, as well as the surrounding environment and community. The biosafety level required in laboratories derives from the characterization of the risk, and is not automatically derived from the risk group to which the pathogenic agent belongs. It is obvious that the biosafety level for a laboratory which cultivates a RG3 agent, will be higher than the level needed for a laboratory which performs diagnostic tests on inactivated biomaterials on the same agent.
Specific checklists, derived from the WHO laboratory biosafety manual, which in Italy are also defined by the National Institute of Labor Safety Insurance (Istituto Nazionale Assicurazione Infortuni sul Lavoro) in its 6th Fascicle published in 2010 [3] are necessary to verify the compliance of a given laboratory with the required biosafety level.

Biosafety in pathology and the actual epidemiological situation

According to the international consensus, ***SARS-CoV-2 has to be classified as a Risk Group 3 (RG3)*** human pathogen [4-7].
SARS-Cov2 can be transmitted through inhalation of aerosol droplets or through contaminated surfaces, where the virus persists viable up to 72 hours on stainless steel and plastic. In our laboratories we can produce aerosol droplets during centrifugation and vortexing of fluids, while surface contamination can occur in a variety of situations, like leakage of fluids during dissection of fresh or inadequately fixed specimens.
SARS-Cov2 virus has been mostly identified in tissue and biomaterials of pulmonary origin, but it can also be identified in other biomaterials, like blood and stools, while the presence of viral material in urine has not been detected or is at most questionable [8-10]. The possibility of oral-fecal transmission is controversial, but has to be taken into account. Therefore, given the extensive and partially unknown prevalence of the infection in the general population, we should considered at high risk of contamination all lung tissues and fluids, but also other specimens, including gastrointestinal specimens [11].
The biomaterials identified as possible sources of contamination in our laboratories are:
1 tissue samples derived from autopsies;
2 surgical and cytological specimens such as:
   - unfixed surgical and cytological specimens, including tissues for frozen sections and specimens collected with formalin-free vacuum technology,
   - inadequately fixed surgical specimens (e.g.: lung specimens floating in formalin jars, any surgical specimen in a jar with insufficient formalin or which has been kept immersed in formalin for a short period of time),
   - fine needle aspiration specimens for which rapid on-site evaluation is performed,
   - cytological samples collected in transporting mediums which do not guarantee viral inactivation,
   - samples for flow cytometry.

All the above described materials should be considered as potentially infective and our laboratories must strictly adhere to biosafety regulation when manipulating these samples. Conversely, all properly fixed tissues and cytological samples are not at risk and can be safely manipulated using standard procedures [12].
In surgical specimens, SARS virus are inactivated by fixation in 10% buffered, neutral formalin at room temperature for 1 day and by alcoholic fixation in 70% ethanol [13]. It is prudent, in the actual epidemiological situation, to perform gross sectioning only after samples have been adequately fixed, avoiding as much as possible manipulation of unfixed/inadequaltely fixed specimens. We also suggest to suspend the use of formalin-free vacuum collection of surgical specimens if the safety level of the laboratory does not guarantee adequate protection.
Manipulation of cytological samples needs more rigid changes in procedure and attention than those required for histological samples, mostly based on the evidence that liquid-based preparations utilize low alcohol concentrations for conservative rather than for fixative purpose.
The recent Laboratory biosafety guidance related to coronavirus disease 2019 released by the World Health Organization, states that *"non-propagative diagnostic laboratory work (e.s: sequencing, nucleic acid extraction) should be conducted in a facility using procedures equivalent to biosafety level 2(BSL-2)"* [14]. This document does not refer directly to surgical pathology activities, but our activities can be clearly considered as "non-propagative". The Centers for Disease Control and Prevention states that all surgical

pathology activities, including molecular analyses, performed on formalin fixed or inactivated samples pertaining to patients affected by COVID-19 should be performed in a BSL-2 laboratory [1]. Among the requirements for a BLS-2 laboratory are: (a) daily decontamination of all work surfaces when work is complete, (b) use of personal protective equipment (PPE), (c) appropriate disposal of contaminated material, (d) prevention of injuries such as cuts and other breaches of the skin and mucous membrane exposures. All procedures that can cause infection from aerosols or splashes are to be performed within a biological safety cabinet (BSC).

What to do in the actual epidemiological SARS-CoV-2 situation

As it has been reported that formalin and ethanol fixation inactivate the virus, we will pragmatically focus here only on some aspects concerning the manipulations of unfixed/inadequately fixed surgical and cytological samples. We will not consider the management of properly fixed samples and the activities related to autopsy practice, which have been addressed by several agencies including The Royal College of Pathologists [5], the European Centre for Disease Prevention and Control [15] and the Italian Society of Surgical Pathology (SIAPEC) [16].

- **Clinical information.** All samples shall be accompanied by adequate clinical information regarding SARS-CoV-2 status. In particular, the case must be identified if: a) positive for SARS-CoV-2, b) suspicious for SARS-CoV-2. Samples of positive and suspicious cases shall be transferred to the laboratory in a secondary disposable biohazard ziplock bag whenever possible.
- **Paperwork.** As the virus can persist on paper at least for 24 h, paperless electronic request transmission should be preferred.
- **Frozen sections.** Frozen sections from patients which are positive/suspicious for SARS-CoV-2 infection are strongly discouraged [6]. This recommendation is particularly important when dealing with materials from the upper and lower airways. Should this kind of activity be absolutely needed, personnel who manipulate the samples shall adhere to strict biosafety criteria: frozen sections should be preferentially done in cryomicrotomes which allow aerosol containment [if not available the cryomicrotomes shall be cleaned and disinfected using alcohol 100°C (to avoid ice formation) after each procedure]. Personnel shall be appropriately instructed and shall always wear appropriate PPE.
- **Biosafety cabinets (BSC) and fume hoods (FH).** All activities implying the use of potentially infectious unfixed/inadequately fixed material should be performed in BSC. BSC protect workers by a) containing vapors, dusts, gases, and fumes moving them as air flows into the hood and then out of the laboratory via the exhaust system, and b) shielding the worker with a clear sliding window that contains aerosols and prevents injury from splashes that may occur inside the hood. There are three classes of biosafety cabinets: Class I, Class II, and Class III. Class I biosafety cabinets provide personnel and environmental protection but no product protection. Class II and Class III cabinets provide personnel, environmental, and product protection. BSC Class II biosafety cabinets are widely used in biological research laboratories and are differentiated into 4 types, labeled as A1, A2, B1, or B2. Any procedure with the potential to generate aerosols or droplets (e.g., vortexing, centrifuging, pipetting) should be performed in a certified Class II BSC. If no certified Class II BSC is available, or if instruments (e.g., centrifuges, analyzers, automated extraction equipment) cannot be used inside a BSC, extra precautions can be used to provide a barrier between the specimen and personnel. Examples of these precautions include using additional personal protective equipment (PPE) (e.g., mask, respirator, face shield) or other physical barriers (e.g., splash shield, centrifuge safety cups, sealed centrifuge rotors) to reduce the risk of exposure to laboratory personnel. BSC class I with appropriate air flow toward external, which are frequently used in surgical pathology laboratories in Italy, are similar to chemical fume hoods, substantially differing from H because of the use of high efficiency particulate air (HEPA) filters: these BSC are appropriate for risk mitigation. Pure FH, without HEPA filters, and at even more importantly without appropriate air flow toward external, are not appropriate for biosafety.
- **Manipulation of unfixed/inadequately fixed surgical specimens.** As stated above we suggest to suspend the use of formalin-free vacuum collection of surgical specimens if the safety level of the laboratory does not guarantee adequate protection. Surgical specimens in formalin jars, may arrive in the grossing room with only partial fixation: these samples have to be manipulated following biosafety procedures. Pragmatically, as fixation is a process which takes time and should be favored by gross sectioning of the organs, we suggest that incompletely fixed samples, especially those of the lungs and gastrointestinal tract, shall be pre-

liminarily grossly sectioned without extracting the samples from the jars followed by direct injection of formalin in the organ/tissue (e.g.: in lung tissue usually floating in the jar), and definitively sampled only after complete fixation. This approach could facilitate formalin fixation (and hence morphological and biomolecular preservation), while minimizing contamination risk.

- **Manipulation of unfixed/inadequately fixed cytological.** Procedures which can produce aerosol shall always performed under high biosafety precautions. Centrifuges should have safety buckets or sealed rotors and should be placed in a BSC. Devices for vortexing tubes should also be placed in a BSC. If the laboratory is not equipped with appropriate BSC, the centrifuges and vortex shall be placed in available chemical fume hoods, possibly with ultraviolet sterilization [1]. It is extremely important to underline that the cytological evaluation of positive/possible SARS-Cov2 samples, especially for those of bronchial/pulmonary origin, should be performed only for extremely necessary cases, possibly limiting and reducing the number of routine samples. The cytological material shall be processed in dedicated BSC under the supervision of specialized technicians wearing adequate protective equipment and whenever possible it is useful to fix the cytological samples which arrive unfixed in the laboratory, using a series of different approaches according to the different cytological samples. All these manipulations shall be done in a BSC. Specifically, for **pulmonary cytological samples** add a 70% (bronchial lavage) to 95% (sputum) alcoholic fixative solution, in a range from 1:1 to 2:1, followed by formalin fixation and paraffin embedding in a cell-block. **Pleural, peritoneal and serous fluids**, after centrifugation and deletion of supernatant, treat with 70% alcoholic fixative solution followed by formalin fixation and paraffin embedding in a cell-block. For **urine**, it is reasonable to add a 95% alcoholic fixation solution in a proportion 2:1. For **liquor**, the possibility of alcohol treatment it is not ideal mostly due to the scant amount of cellular material in the sample. While, this approach may slightly alter the quality of the sample, especially when compared to those usually processed with methanol solution, it may be wiser to modify the standard approach to guarantee safety in the laboratory, which is the main issue in this dramatic emergency condition.
- **Manipulation of liquid based cytological samples (LBC)**. These cytological samples are usually collected and transferred to the surgical pathology laboratory in liquid mediums, however the alcoholic concentration may not be high enough for viral inactivation. Therefore, these samples have to be manipulated with high biosafety precautions. For example, for non-cervical *samples processed with Hologic technology* (Marlborough, Massachusetts, US), we suggest the adoption of completely modified "off-label" method based on the collection of cytological material in a 70% ethyl alcohol solution instead of the Cytolyt® (Hologic) solution, followed by centrifugation at 600g for 10 minutes (or 1200g for 5 minutes). The next step is to discharge supernatant and resuspend cell pellet adding 30 ml of Cytolyt® solution. This phase can be followed by another centrifugation at 600g for 10 minutes, with discharge of the supernatant. Finally, it is necessary to add the specimen to the PreservCyt® solution vial, followed by PreservCyt® fixation for 15 minutes; the specimen can now be processed on a ThinPrep processor [17]. Cervical cancer screening was stopped in Italy during the COVID-19 emergency: therefore, cervical LBCs should not be an issue for cytology laboratories. However, since the alcohol concentration in the most widespread LBC technologies may not reach 70%, sporadic pap tests may be preferentially done by non-LBC technique, and immediately fixed in 95% alcohol after preparation. If LBC cervical specimens are sent to our laboratories, they have to be manipulated following all biosafety roles.
- **Personal protection equipment.** Personnel which manipulates surgical and cytological samples (unfixed/inadequately fixed), shall always wear a) FFP3 mask (or, if this kind of mask is not available, a FFP2 mask), b) eye and face protection, c) double gloves and d) waterproof scrub. Personnel shall also be instructed how to wear and dispose PPE and must always wash hands when moving from one area of the laboratory to another.
- **Decontamination.** SARS-CoV-2 virus can persist on a variety of surfaces for different periods of time: it has been shown that it can persist up to 72 hours on steel and plastic, and is usually non-viable after 24 hours on cardboard [18]. Therefore, all surfaces and equipments (e.g.: centrifuges, vortex, BSC, grossing surfaces, cryostats) shall be decontaminated using appropriate products. Recently prepared 0.1%-0.5% Sodium hypochlorite solution, 70% ethanol or 0.5% hydrogen peroxide are all adequate for disinfection. UV disinfection of cabinets is useful, but it has to be pointed out that it may not be efficient when the cabinets contain several objects. A manual spray device can be very useful to reach every angle of cabinets or cryostats.

Conclusion

The present epidemiological situation highlights the importance of biological risk management in surgical pathology. All fixed samples and all paraffin blocks are at extremely low (negligible or absent) risk of coronavirus infectivity as it has been reported that formalin fixation and heath exposure (in the range of the temperature for paraffin embedding of tissues) inactivate most coronaviruses [12,13,19]. However, we are faced daily with manipulation of unfixed/inadequately fixed samples which requires strict adherence to biosafety rules. The widespread use of formalin-free vacuum technology has greatly increased the number of unfixed samples which are manipulated in our laboratories, increasing the need of appropriate risk management approach. This poses important issues of biosafety which require immediate action by our community.

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Guidelines

Management of the corpse with suspect, probable or confirmed COVID-19 respiratory infection – Italian interim recommendations for personnel potentially exposed to material from corpses, including body fluids, in morgue structures and during autopsy practice

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Introduction

COVID-19 (Coronavirus Disease-19) is the most urgent health emergency worldwide and all professionals are called to give support in the diagnosis and treatment of patients affected by this disease.

The Scientific Society of Hospital Legal Medicine of the National Health System (COMLAS) and the Italian Society of Anatomical Pathology and Cytology (SIAPEC) produced this document with the intent of offering

a technical support to professional involved in the autoptic activities during the **Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2**) epidemic infection.

Coronaviruses are a broad category of viruses known to cause diseases ranging from the common cold to Middle Eastern Respiratory Syndrome (MERS) and Acute Severe Respiratory Syndrome (SARS). Recently, a novel coronavirus (nCoV) was identified that had never previously been found in humans [1-3].

The viruses responsible for SARS include **SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2)**; the disease caused by the new Coronavirus is called **COVID-19 (Coronavirus Disease-19)** and represents a cluster of pneumonia that has spread to China and other countries of the world since 31 December 2019 [4-8].

Initial reports suggest that transmission occurs during close contact with an infected subject, mainly through respiratory droplets produced in the act of speaking, coughing or sneezing [9,10]. Droplets can settle on the eyes, nose or mouth, as well as be inhaled by people nearby. At present, long-distance aerial transmission from person to person is unlikely.

Common signs of infection include fever, cough and breathing difficulties. In severe cases, the infection can cause pneumonia, severe acute respiratory syndrome, multi-organ dysfunction or failure, and death [11-16].

WHO, CDC and ECDC have provided clear details on measures to prevent the spread of the virus [17-21]. Standard recommendations to prevent the spread of infection include regular hand washing, covering the mouth and nose when coughing or sneezing and avoiding close contact [22,23].

At the Italian level, the extra- and intra-hospital path of suspect or confirmed cases of COVID-19 and the indications relating to the use of personal protective equipment (PPE) by healthcare personnel are extensively defined [24-28].

In view of the limited scientific knowledge and evidence on SARS-CoV-2 infection, it is therefore considered appropriate to proceed systematically with the management of COVID-19 cases [29,30], whether they are suspect, probable or confirmed, accepted at the morgue. Hence the need to condense the available evidence on biological risk, individual protection and autopsy investigation with the aim of providing a practical guidance.

The recommendations expressed in this document have been developed to help healthcare professionals and morgue staff manage COVID-19 deaths by advising on possible risks and preventive measures. In the same way, indications will be provided about the post-mortem investigation of suspect, probable or confirmed cases of COVID-19.

Purpose and field of application

The classification of infectious hazards needs to be periodically reviewed and updated in light of global epidemiological trends. The categorization of infectious biological agents provides for the attribution to hazard groups (Hazard Group 1-4) based on the probability of causing disease in humans, the probability of spreading the infection in the community, the availability of prophylactic or therapeutic measures [31].

The Advisory Committee on Dangerous Pathogens (ACDP) in early 2020 established a provisional classification of SARS-CoV-2 as an HG3 pathogen (Hazard Group 3). In this regard, it should be emphasized that HG3 agents can cause serious diseases in humans and constitute a serious risk for professionals; the agent may spread in the community, but effective prophylactic or therapeutic measures are usually available. The risks for personnel operating in the morgue facilities, in most infections, are minimal when standard universal precautions for infection prevention are applied [32-35].

The purpose of the operating procedure is, therefore, to indicate how to manage the bodies of suspect or confirmed cases of COVID-19 in order to minimize the risk of environmental contamination and the contagion of personnel involved in the process. According to this perspective, the document is intended to recommend:

- the preparation of standard operating procedures (SOP) for the containment of infectious risk;
- adequate conduct for carrying out activities and maneuvers at risk during autopsy checks on cases of SARS-CoV-2 infection;
- personal protective equipment (PPE) to be used in daily practice;
- the optimal evaluation pathways for the diagnosis of SARS-CoV-2 infection.

The present document is mainly aimed at staff serving in morgues (health professionals, trainees, as well as auxiliary, administrative and surveillance staff) and must be applied to every death from COVID-19, whether suspect, probable or confirmed.

Terms and definitions

COVID-19 case definition

The case definition is based on the information currently available and can be updated based on the evolution of the epidemiological situation and the scientific knowledge available [36].

The Circular issued by the Italian Ministry of Health on 09.03.2020 provides - in updating and replacement of the previous ones - the definition of suspect, probable and confirmed case [37].

Suspect case

Laboratory investigations for COVID-19 should be performed for suspect cases according to the following criteria, based on the updated WHO and ECDC definitions:

- patient with acute respiratory tract infection (sudden onset of at least one symptom between fever, cough, and dyspnea) with no other etiology that fully explains the clinical presentation and with a history of travel or residence in a country or area that has reported local transmission or community in the 14 days preceding the onset of symptoms (consult the WHO reports on the situation; www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/), *or*;
- patient with any acute respiratory infection who has been in close contact with a probable or confirmed case of SARS-CoV-2 infection in the last 14 days before the onset of symptoms, *or*;
- patient with a severe acute respiratory infection (fever and at least one symptom or sign of respiratory diseases such as coughing and difficulty breathing) who requires hospitalization (SARI), with no other etiology that fully explains the clinical presentation, *or*;
- in the context of primary care or in the hospital emergency room, all patients with symptoms of acute respiratory infection must be considered suspect cases if the local transmission has been reported in that area or in the country.

Any positivity found for common respiratory pathogens may not exclude co-infection with SARS-CoV-2 and therefore samples must be tested for this virus anyway.

Probable case

Suspect case for which the result of the test carried out at the Reference Laboratories using specific Real Time PCR protocols for SARS-CoV-2 is doubtful or not conclusive, or, for which positivity to the pan-coronavirus test is confirmed.

Confirmed case

Case with ascertained positivity for SARS-CoV-2 infection, regardless of the presence of clinical signs and symptoms.

Definition of close contact

Close contact with a probable or confirmed case is defined as:

- person living with a case of COVID-19;
- subject who had physical contact with a COVID-19 case (e.g. handshake);
- subject who had unprotected direct contact with secretions from a COVID-19 case;
- subject who had direct contact with a COVID-19 case at a distance of less than 2 meters and for a time greater than 15 minutes;
- subject who has been in a closed environment (e.g. classroom, meeting room, waiting room, *etc*.) with a COVID-19 case for more than 15 minutes and at a distance of less than 2 meters;
- healthcare professionals or other subjects providing direct assistance for a COVID-19 case, or laboratory operator handling specimens of a COVID-19 case without the recommended personal protective equipment (PPE);
- aircraft contact with a case of COVID-19 sitting in the same row or in the two preceding or subsequent rows, traveling companions or assisting persons and crew members serving in the section of the aircraft where the case was housed (if the case manifests serious symptoms or has made movements inside the aircraft, the passengers seated in the whole section or all the passengers of the aircraft can be considered close contacts).

The epidemiological link can be established if contact is documented between the suspect case under consideration and a confirmed case within a period of 14 days before the onset of symptoms.

Hygiene precautions in the management of suspect, probable or confirmed case of COVID-19

In compliance with scientific evidence and the provisions of the Italian Ministry of Health, all health professionals involved in the management of suspect or documented cases of COVID-19 are required to adopt, in addition to standard safety measures, the necessary precautions to prevent the transmission of the virus [38,39].

Hand hygiene

Carry out antiseptic hand washing with alcoholic solution or antiseptic soap and water after each contact with the body.

The antiseptic hand washing technique must comply with the WHO sequence [40,41]. Failure to comply with proper hand hygiene negates the protective efficacy of personal protective equipment.

Contact precautions

In addition to the standard precautions, anyone who comes into contact with a suspect case of COVID-19

must strictly adhere to the contact precautions.
Is essential:

- to pay close attention to avoid accidentally touching with the face (eyes, nose, and mouth);
- to minimize the number of people present at the same time in the area where the body is allocated;
- to always close the door of the room where the body is located and keep openings to a minimum.

Individual protection measures

PPE offers the highest possible protection against HG3 infectious agents. The morgue staff must use the following PPE in the coded manner for the different operational phases of body management (acceptance, custody, handling and autopsy) (Tab. I):

- disposable headgear;
- double pair of disposable gloves;
- cut-resistant protective gloves;
- respiratory filter FFP2 or FFP3 (the latter recommended in the procedures that produce aerosols) and face protection (goggles or protective visor);
- disposable long-sleeved gown or waterproof suit;
- disposable overshoes.

Dressing procedure

It must take place in the filter area according to the following steps:

1 Remove all jewelry
2 Check the integrity of the personal protective equipment
3 Put on disposable headgear and overshoes
4 Put on the first pair of disposable gloves
5 Put on the disposable gown, fastening it on the neck and hips, or waterproof suit
6 Wear FFP2 facial filter (FFP3 filter in case of procedures that produce aerosols)
7 Wear protective goggles and/or visor
8 Put on the second pair of disposable gloves

The main stages of the dressing procedure are summarized below on the basis of the recommendations of the CDC on the correct use of personal protective equipment [42].

Correct use of facial filter

1 Check the integrity of the device
2 Open the ends of the lower flaps of the mask, making sure that the valve (FFP3) is correctly oriented
3 Turn the mask upside down allowing the elastics to come out
4 Slightly bend the upper part of the mask forming a "V" to favor a better dressing
5 Remove the adhesive tab (if present)
6 Put on the mask by adjusting the elastic bands with both hands
7 First place the upper elastic in the middle of the back surface of the head, then the lower elastic on the nape
8 Place the mask under the chin and shape the upper part to allow adequate adherence to the face
9 Perform the mask tightness test. Cover the facial

Table I. Specific risks and recommended PPE for the different operational phases of body management.

Phase	Risks	PPE
Admission and Handling	- Contact with potentially infected material from corpses	- Disposable gloves (single pair) - Respiratory filter FFP2 - Goggles or protective visor - Disposable long-sleeved gown or waterproof suit - Disposable overshoes
Swab collection	- Contact with potentially infected material from corpses	- Disposable gloves (single pair) - Respiratory filter FFP2 - Goggles or protective visor - Disposable long-sleeved gown or waterproof suit - Disposable overshoes
Autopsy investigation	- Contact with potentially infected material from corpses - Sharp injuries - Production of splashes and aerosols	- Disposable headgear - Disposable gloves (double pair) - Cut-resistant protective gloves - Respiratory filter FFP3 - Goggles or protective visor - Disposable long-sleeved gown or waterproof suit - Disposable overshoes
Environmental disinfection	- Contact with potentially infected material from corpses - Production of splashes and aerosols	- Disposable gloves - Respiratory filter FFP3 - Goggles or protective visor - Disposable long-sleeved gown or waterproof suit - Disposable overshoes

filter with both hands and perform an inhalation or exhalation test, as described below:
- perform a deep inhalation; if the mask collapses slightly, the tightness is adequate; if air is perceived from the edges of the mask, better adjust the position and length of the elastics; if an air leak is felt around the nose, correctly position the upper support, the patch, and the nose clip;
- perform a forced exhalation; if there is no air leak, the mask is properly sealed.

Undressing procedure

At the end of the procedures, in the filter areas, it is essential to:
- avoid touching any surface before carrying out the undressing procedure;
- avoid any contact between potentially contaminated PPE and the face, mucous membranes or skin.

The undressing procedure must take place in the filter area, taking care to avoid self-contamination, respecting the following sequence:
1 Remove the disposable gown and the overshoes by disposing of them in a special container
2 Remove the first pair of gloves and dispose of them in a special container
3 Remove the protective glasses and sanitize them
4 Remove the facial filter, taking care not to touch the front surface of the mask (remove it from the elastics with back-forward movement) and dispose of them in a appropriate container
5 Remove the headgear
6 Remove the second pair of gloves and dispose of it in the special container
7 Perform antiseptic hand washing

The main stages of undressing are illustrated below in two different sequences, in accordance with the recommendations of the CDC on the correct use of personal protective equipment [42].

Before leaving the autopsy rooms, carefully remove the PPE to avoid contamination and dispose of it in the appropriate containers.

Any reusable PPE (goggles, visors, respirators, etc.) must be properly cleaned, decontaminated and kept in view of subsequent use.

After the undressing procedure, scrub the hands with soap and water for at least 20 seconds. If the hands are not visibly dirty and there is no running water available, an alcoholic solution with a concentration of 60-95% can be used. However, in the event that the hands are visibly dirty, always scrub with soap and water before using any type of disinfectant.

Avoid contact of face, mouth, and eyes with gloved or unwashed hands [43].

Ensure the availability of hand hygiene devices in the immediate vicinity of the PPE removal area.

Operating procedures

Operating procedure for the management of the corpse with suspect, probable or confirmed diagnosis of COVID-19

The proposed procedure is aimed at the safe management of the phases of acceptance, handling, custody, and discharge of the body with suspect, probable or confirmed diagnosis of COVID-19 [44-47]. The objective has been pursued by drawing up the following recommendations:
- the acceptance and handling of the body must be done by personnel equipped with the recommended PPE;
- the body must be positioned on a sanitized metal stretcher for custody and subsequent investigations;
- at the end of the investigations, the body must be placed in the coffin with clothes and wrapped in a sheet soaked in disinfectant solution;
- if the stay of the corpse in the morgue is necessary - pending or at the conclusion of the investigations - the same must take place inside a special closed body bag and dedicated refrigerated room;
- at the end of the handling and transport operations, all the equipment used must be subjected to sanitization.

Recommendations for autopsy investigation in cases of suspect, probable or confirmed COVID-19

For the safe and effective performance of HG3 (Hazard Group 3) autopsy investigations, is required (Fig. 1):
- generic risk assessment and adoption of universal standard precautions;
- knowledge of possible pathological findings that can be highlighted;
- the definition of SOP (Standard Operating Procedures) for the management of autopsies with high biological risk.

The use of universal precautions effectively protects against most risks related to SARS-CoV-2 infection. Professionals have a duty to carry out risk assessment for each case in order to prevent actions that could put operators at risk.

Pre-autoptic risk assessment should include:
- anamnestic information collected from close relatives or acquaintances;
- information from family physicians;
- information obtained from healthcare facilities.

Information on the circumstances of the death is essential. In addition to information on the state of health

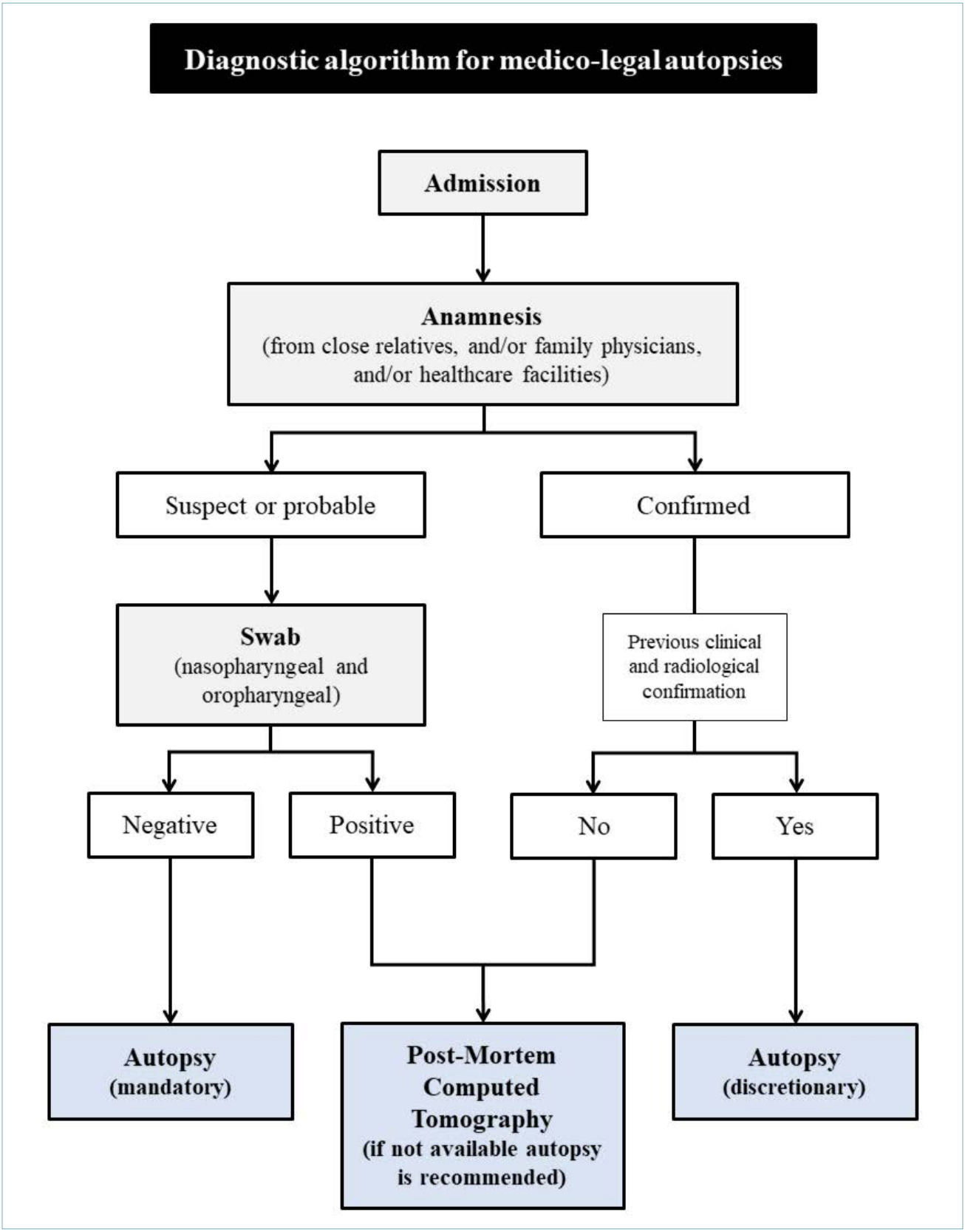

Figure 1. Diagnostic algorithm for medico-legal autopsies.

and place of death, knowledge of any previous national and international trips, as well as laboratory data (positive and negative), is fundamental. It is important not to assume that the information acquired is accurate.
The criteria for the preliminary assessment of deaths and the definition of any cases are the same as those used to evaluate the possible risk of infection in living patients.
If after preliminary assessment it is believed that a death may be due to COVID-19, the subsequent investigations must be oriented towards confirmation of diagnosis and precise definition of the role of SARS-CoV-2 infection in the determinism of death (study of any pre-existing conditions and comorbidities capable of characterizing a condition of fragility of the subject). Judicial autopsies are an exception as any diagnostic activity depends on the prior consent of the Prosecutor.
For suspected or probable HG3 infection, preliminary nasopharyngeal and oropharyngeal swabs are recommended for diagnostic confirmation. In the event of a positive swab, especially if there is clinical, laboratory and radiological confirmation, the execution of the autopsy investigation is discretionary. In cases of swab positivity in which there is no clinical or radiological confirmation of the diagnosis, the study using post-mortem computed tomography (PMCT) is recommended in accordance with the availability and the care loads on the hospital structure; if a post-mortem radiological study is not possible, an autopsy should be performed. If the swab is negative, autopsy is mandatory.
The autopsy must concern all body districts and be oriented towards the search for significant findings for diagnosis [48-50]. For this purpose, en bloc extraction of the cervical and thoracic organs is recommended in order to adequately define the respiratory pathological manifestations and facilitate sampling operations.
In general, the following measures are recommended for the safety of autopsy practice:

- mantain all the necessary equipment on hand to avoid leaving the autopsy area to find additional items;
- in order to prevent accidents, limit the phases in which the intervention of multiple operators is foreseen and privilege the activity of expert operators;
- use PM40 scissors or blades with blunt tips, in order to reduce the risk of injury;
- minimize the presence of cutting edges in the work area; their position must be known to all operators at all times;
- cut the organs not fixed in formalin keeping them still on the table and using a sponge, paying attention to protect the hands;
- use the oscillating saw with aspiration of the bone aerosol for the opening of the cranial cavity; in case of unavailability use a hand saw wearing metal mesh gloves;
- avoid incongruous maneuvers for the disposal of needles after sampling; needle and syringe must be placed in the appropriate container for cutting edges;
- maintain adequate water pressure to avoid splashing;
- clothing worn by the corpse must be disposed of as contaminated special waste;
- prepare a register to record the specific activities carried out (cadaveric inspection, autoptic assessment, collection of samples, etc.), the date of performance of the same, as well as the names of the operators directly involved and of the staff present in the morgue structure during the activity.

The team performing an autopsy at high infectious risk should ideally include an operating forensic pathologist/pathologist, a “clean” assistant forensic pathologist/pathologist and a technician. The assistant forensic pathologist/pathologist performs auxiliary tasks such as sample management.
Residents should be involved in autoptic activity only under the supervision of senior staff, but especially when they have demonstrated knowledge of the risks, awareness of protective measures and proven experience in autopsy practice [51,52]; briefly, for HG3 cases, the Authors’ recommendation is to limit the involvement of residents in the most risky procedures such as evisceration.
From a structural point of view, performing HG3 autopsies requires effective ventilation in the autopsy room and the possibility to maintain an adequate distance in carrying out the different activities [53].
In accordance with the CDC indications, autopsies in cases of suspect or confirmed COVID-19 are always practicable as long as the conditions of maximum safety and infectious disease protection for operators and work environments can be guaranteed.
In particular, the CDC reports that “Autopsies on decedents with known or suspected COVID-19 should be conducted in Airborne Infection Isolation Rooms (AIIRs). These rooms are at negative pressure to surrounding areas, have a minimum of 6 air changes per hour (ACH) for existing structures and 12 ACH for renovated or new structures, and have air exhausted directly outside or through a HEPA filter. Doors to the room should be kept closed except during entry and egress. If an AIIR is not available, ensure the room is negative pressure with no air recirculation to adjacent spaces.
A portable HEPA recirculation unit could be placed in

the room to provide further reduction in aerosols. Local airflow control (i.e., laminar flow systems) can be used to direct aerosols away from personnel. If use of an AIIR or HEPA unit is not possible, the procedure should be performed in the most protective environment possible. Air should never be returned to the building interior, but should be exhausted outdoors, away from areas of human traffic or gathering spaces and away from other air intake systems" [54-55].

Recommendations for the safe collection and transport of samples

The present document provides specific guidance for the collection and management of cadaveric specimens for the diagnostic in-depth of COVID-19. Similarly, recommendations are made for biosafety and infection control during specimen collection and handling [55,56].

In cases where an autopsy is performed, the collection of the following samples for SARS-CoV-2 research is recommended:

- upper respiratory tract swabs (nasopharyngeal swab and oropharyngeal swab);
- swabs of the lower respiratory tract (from each lung);
- organ and tissue samples to be kept in formalin for subsequent histopathological investigations.

If the investigation ends with the cadaveric inspection, only the collection of swabs from the upper respiratory tract is recommended.

It is important to keep the SARS-CoV-2 samples separate from the samples taken for other microbiological and non-microbiological investigations.

The presence of health professionals in autopsy rooms should be limited to operators engaged in sampling. Since the collection of cadaveric samples does not induce coughing or sneezing, a negative pressure environment is not required.

Personnel must comply with the standard precautions previously described.

Use only synthetic fiber swabs with plastic rods; different devices with wooden barrels or with calcium alginate can inactivate the viral agent and make the PCR test ineffective [57,58].

For the proper collection of a nasopharyngeal swab:

1 insert a swab into the nostril parallel to the palate;
2 leave the swab in place for a few seconds to absorb secretions;
3 perform a contralateral swab in the same way and with the same device.

Proper execution of the oropharyngeal swab requires:

1 insertion of the device into the oral cavity until it reaches the posterior pharyngeal wall (attention to avoid contact with the tongue);
2 leave the swab in place for a few seconds to absorb secretions.

To perform an adequate lower respiratory tract swab:

1 insert the swab into the branches of the main bronchus;
2 leave the swab in place for a few seconds to absorb secretions;
3 perform a contralateral swab in the same way but with a different device.

Immediately after the procedure, place each of the swabs in a different sterile tube containing 2-3 ml of transport medium for subsequent storage in the refrigerator at 2-8°C before processing. Each container must be labeled indicating the identification number of the subject, the identification code of the sample, the type of sample and the date of collection of the same.

Safe preparation of specimens for transport involves:

1 insertion of sterile tubes containing the swabs into a secondary container;
2 placing the containers inside a sealable clean plastic bag;
3 if possible, the insertion of the sealable bag in an additional bag for biological samples;
4 transfer outside the autopsy area and delivery to an operator equipped with disposable nitrile gloves for transport.

Regarding the sampling of organs for formalin fixation [59,60], the collection of tissue samples with a size of 4-5 mm thickness is recommended. The volume of formalin used for fixation should be 3 times greater than the volume of the tissues. Use 10% buffered formalin for at least 48 hours to achieve optimal fixation. With respect to standard samples for histological investigations, particular care must be taken with a sampling of the trachea (proximal and distal) and lung (the hilar region with segmental and primary bronchi, as well as parenchyma representative of all the lobes of both lungs).

This sampling method finds its rationale in the viral tropism for the epithelia of the upper respiratory tract (in particular primary airways and segmental bronchi) which for this reason exhibit greater performance in molecular tests and immunohistochemical investigations [61,62].

Further sampling should be guided by the anamnesis, as well as by the autopsy findings and may include, for example, samples for bacteriological culture tests or toxicological investigations.

Post-procedural prophylactic rules and environmental disinfection

Below are the general guidelines for cleaning and disposal of waste following a necropsy investigation of a suspect or confirmed case of COVID-19 [63,64]; it should be noted that at present the persistence time of SARS-CoV-2 on surfaces is uncertain [65,66].

At the end of autopsy investigations, the body must be positioned in a body bag and transported in a refriger-

ated room. Disinfect the outside of the body bag with a hospital disinfectant applied according to the manufacturer's recommendations. It is also recommended to this phase to use suitable PPE by each operator involved in the movement and exit phases of the body.
In addition, following an autopsy on a subject with suspect or confirmed COVID-19, the following recommendations for disinfection of autopsy rooms should be applied:

- keep ventilation systems active during cleaning;
- wear disposable gloves when cleaning and handling cleaning or disinfectant solutions;
- dispose of gloves after cleaning; do not wash or reuse the gloves in any case;
- use eye protection, such as a visor or goggles, if splashing is expected;
- if necessary, use respiratory protection based on the type of detergent or disinfectant;
- wear a long-sleeved waterproof device to protect skin and clothing;
- use disinfectants with indications of efficacy against human coronaviruses;
- clean the surfaces and apply the disinfectant ensuring an adequate contact time for effective disinfection;
- comply with the safety precautions and warnings indicated on the product label (for example, allow adequate ventilation in restricted areas and ensure correct disposal of the unused product or used containers);
- avoid product application methods that cause the production of splashes or aerosols.

Regarding environmental disinfection, the available evidence has shown that coronaviruses are effectively inactivated by adequate sanitization procedures that include the use of common hospital disinfectants, such as sodium hypochlorite (0.1-0.5%), ethanol (62-71%) or hydrogen peroxide (0.5%). There is currently no evidence to support a greater environmental survival or a lower sensitivity of SARS-CoV-2 to the aforementioned disinfectants [67].
Hard and non-porous surfaces can be cleaned and disinfected as previously described.
Handle with gloves and disinfect properly after use, equipment such as cameras, telephones and keyboards, as well as all objects that remain in the autopsy room.
Cleaning activities must be supervised and periodically checked to ensure that correct procedures are followed. Sanitation personnel must be properly trained and equipped with suitable PPE.
After cleaning and removing the PPE, wash the hands immediately. Avoid touching the face with gloved or unwashed hands.
Environmental disinfection must include cleaning with water and detergent soap on all vertical and horizontal surfaces, followed by disinfection with hospital disinfectants effective against SARS-CoV-2.
For environmental decontamination, it is necessary to use dedicated or disposable equipment. Reusable equipment must be decontaminated after use with a chlorine-based disinfectant. The use of special trolleys is strongly recommended, different from those used for cleaning common areas.
The instruments used for autopsies should be autoclaved or treated through chemical sterilizers.

Management of access and movement of visitors within the facility

Conclusively, it is necessary to formulate recommendations regarding the management of access and movement of visitors within the morgue area. Specifically, it is essential to establish procedures for monitoring, managing and informing all visitors:

- educate visitors on the usefulness of hand hygiene and standard precautions, especially in common areas;
- invite visitors to stay in the facility for the time strictly necessary, avoiding stops and movements in non-essential areas;
- inform visitors about the appropriate use of PPE according to local policies for access and circulation in the facility;
- actively evaluate all visitors for fever and respiratory symptoms at the entrance; if present, visitors should not be allowed access to the facility;
- limit access points;
- encourage the use of alternative mechanisms for interactions with administrative offices;
- schedule the discharge of the dead bodies in order to avoid the coincidence of multiple exits;
- install physical barriers in the access and reception areas to limit close contact with visitors;
- allow access to administrative rooms and exhibition halls in the manner imposed by their size and turnout with the aim of avoiding gatherings and allowing compliance with the interpersonal safety distance of at least one meter;
- limit access to funeral services, allowing persons to stay for the time strictly necessary for administrative needs as well as for the preparation and transport of the body;
- allow access to the ministers of worship for the time strictly necessary for the blessing of the body; suspend any further ceremonial rite.

Autopsy of inpatient subjects

Most of the autopsies performed by pathologists are requested by clinicians with the aim to clarify or confirm the causes of death of inpatients. By correlating clinical data with morpho-histological alterations of different organs and tissues the pathologists draw an epicrisis.
In the case of death for SARS-COV-2 the forefront diagnosis of the infection is based on the detection of the virus on nasal and oropharyngeal swabs with PCR technique. Lung involvement is paramount in SARS-CoV-2 infections and Computerized Tomography (CT) is the routine imaging modality to diagnose and monitor patients with SARS-COV-2 pneumonia [68]. CT may assist in the early diagnosis of lung alterations typical of SARS-COV-2 during screening of patients with highly suspicious conditions, in particular patients with an initial negative RT-PCR screening result [69].
The histopathological pattern of early SARS-COV-2 infection in the lung has been described [14] as an incidental finding on specimens obtained from patients operated for lung cancer, for whom the infection was not diagnosed at the time of surgery. The histological finding of advanced infection phase was reported on biopsy samples obtained from lung, liver and heart [15].
The pathological characteristics of COVID-19 closely resemble those observed in SARS coronavirus and Middle Eastern respiratory syndrome (MERS) [16,70].
The above reported data suggest that in SARS-COV-2 infection the histological examination does not have a diagnostic role in the first instance but confirms the result of the laboratory test and of imaging.

Autopsy of subjects with, suspect, probable or confirmed SARS-COV-2 infection

If an autopsy of inpatient subjects with, suspect, probable or confirmed SARS-COV-2 infection is requested **to diagnose the cause of death**, the procedure must be performed by the pathologists following the above reported operating procedures, including the procedure to collect tissue specimens (Fig. 2).
If the collection of tissue samples on the corpse of a subject with a full picture of infection or suspect of being infected with SARS-COV-2 is considered essential for **disease diagnosis,** an option is also to collect specimens from multiple organs (lung, liver, skeletal muscle) using core biopsy sampling, albeit with the limits of such procedures performed on cadaver (Fig. 2). The specimens must be immediately fixed in buffered formalin for no less than 48 hours.
The post-mortem biopsy sample collection must be: (a) requested on the advice of a multidisciplinary team that includes at least one clinician and one radiologist; (b) agreed with the reference pathologist; (c) the request must report patient personal data; outcome of swabs for COVID-19; clinical /anamnestic data and imaging report.
In the case of specific research protocols that require molecular or immunohistochemical analyses for which it is necessary to proceed with the collection of samples taken and stored in ways other than those indicated (for example, fresh or frozen samples) it is mandatory to agree with the regional reference center that will take care of the procedures for collecting, storing and transporting the material.

Autopsy on patients without apparent SARS-COV-2 infection and for clinical reasons independent of pulmonary problems and/or complications

In the event that a diagnostic autopsy is required for clinical reasons independent of lung pathology and/or complications in subjects who apparently do not have SARS-CoV-2 infection, it is recommended:

- to discuss with the clinicians the clinical and imaging findings and the reason why the postmortem examination is requested;
- to perfom within 2 hours of death an oropharyngeal swab to evaluate the presence of SARS-CoV-2 infection;
- to obtain within 24 hours the result of the oropharyngeal swab. If the result of the swab is not available autopsy cannot be perfomed.

These precautions are necessary in order to preserve the safety of the health workers involved in the procedure and the quality of the diagnosis, responding to the clinical question by focusing on the real reasons for which the postmortem examination was requested. In addition, **colleagues working in the Northern part of Italy in the areas with a greater spread of infection, who performed autopsy of patients with negative swab described histological changes consistent with those of lungs specimens obtained from autopsy of positive patients (necrosis microfoci, thrombosis of small vessels, hyperplasia of second-order pneumocytes, polynucleations and nuclear cytopathic alterations affecting endothelium and pneumocytes).**
Thus, considering the current spread of the disease throughout the national territory, regardless of the hospital structure where the autopsy is carried out, but certainly in all those structures with a high number of positive COVID-19 inpatients, the execution of the diagnostic autopsy in any case must assume that the body is potentially infected and therefore take all appropriate precautionary measures as **indicated in the section "Hygiene precautions in the management of suspect, probable or confirmed case of COVID-19"** so

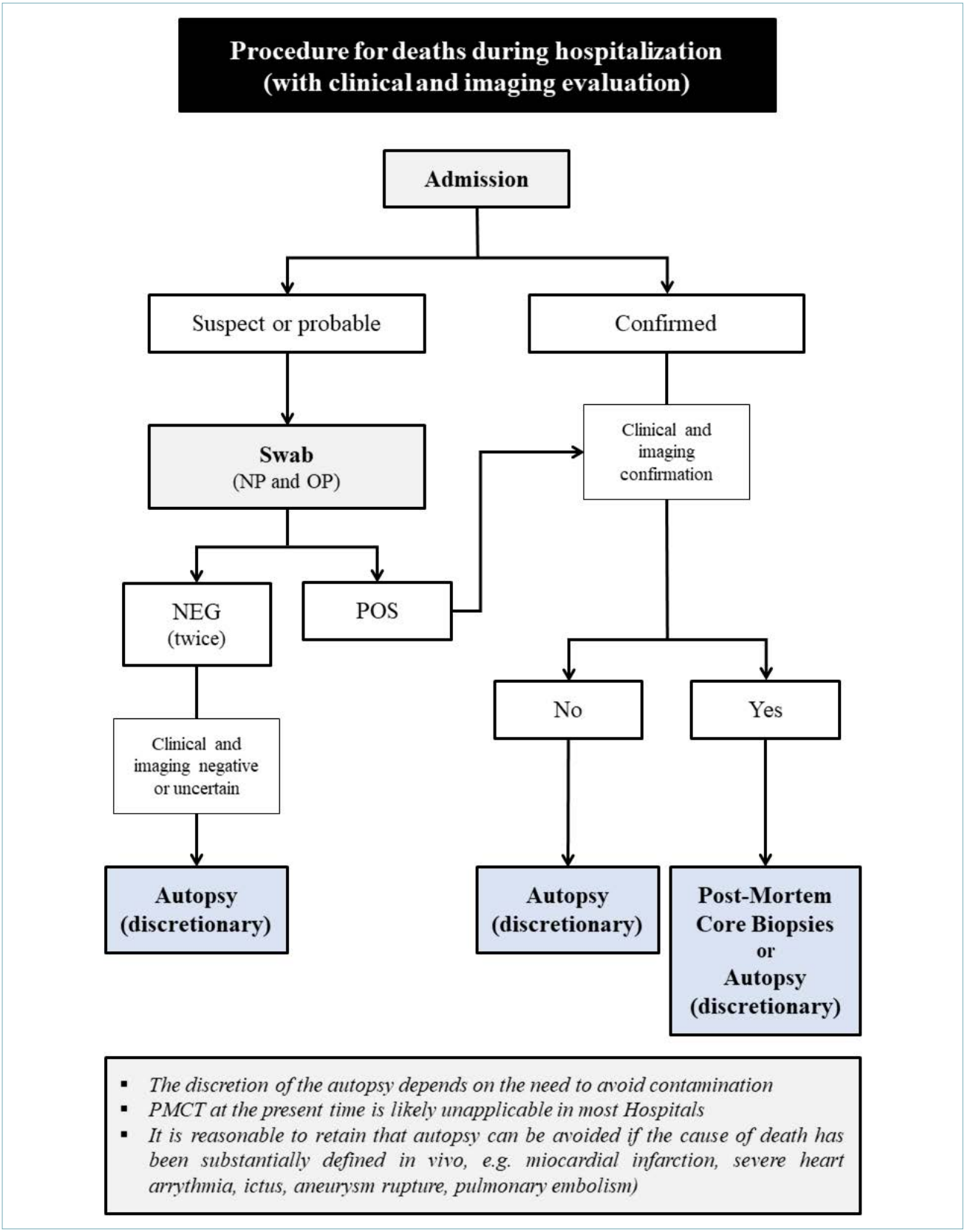

Figure 2. Diagnostic algorithm for hospitalized patients who died and with clinical and imaging evaluation.

as to avoid any risk of contagion, not necessarily from SARS-CoV-2. Consequently, all the procedures indicated for sanitizing the anatomical table and the sector environment must be followed after performing the autopsy.

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Letter to Editor

COVID-19 and management of the corpse

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Dear Editor, we found that the publication on "Management of the corpse with suspect, probable or confirmed COVID-19 respiratory infection - Italian interim recommendations for personnel potentially exposed to material from corpses, including body fluids, in morgue structures and during autopsy practice" is very interesting [1]. In fact, the management of dead body is an important issue but rarely mentioned. There is a risk of possible disease transmission from corpse to practitioner or other persons who come into contact with the corpse. The first possible case of COVID-19 transmitted from corpse by Sriwijitalai and Wiwanitkit is good evidence for the urgent need to implement good protection of disease from dead body [2].

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Review

The broad landscape of follicular lymphoma: Part II

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Summary

Follicular lymphoma is a neoplasm derived from follicle center B cells, typically both centrocytes and centroblasts, in variable proportions according to the lymphoma grading. The pattern of growth may be entirely follicular, follicular and diffuse and rarely completely diffuse. It represents the second most common non-Hodgkin lymphoma, after diffuse large B-cell lymphoma and it is the most common low-grade mature B-cell lymphoma in Western countries. In the majority of cases, follicular lymphoma is a nodal tumor, occurring in adults and is frequently associated with the translocation t(14;18)(q32;q21)/IGH-BCL2. However, in recent years the spectrum of follicular lymphoma has expanded and small subsets of follicular lymphoma, which differ from common follicular lymphoma, have been identified and included in the current 2017 WHO classification. The aim of our review is to describe the broad spectrum of follicular lymphoma, pointing out that the identification of distinct clinicopathological variants of follicular lymphoma is relevant for the patient outcomes and treatment.

Key words: follicular lymphoma, B-cell, centrocyte, centroblast

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Introduction

Follicular lymphoma (FL) is the most common low-grade mature germinal center B-cell lymphoma in Western countries, representing 20% to 30% of all non-Hodgkin lymphomas [1]. The updated 2017 World Health Organization (WHO) Classification includes critical aspects about FL [1]. In recent years, histological and clinical spectrum of germinal center derived B-cell neoplasms has expanded, leading to the conclusion that FL represents a far more heterogeneous entity than originally appreciated. In the previous review we illustrated FL variants encountered in diagnostic practice [2]. Surgical pathologists and hematopathologists should be aware of the broad FL landscape, in order to avoid diagnostic pitfalls and get to more accurate diagnosis.

Although FL is mostly a nodal disease, it can involve primarily extranodal sites. In the current WHO classification, it is well recognized that FL aris-

ing at particular extranodal sites (i.e. duodenum, skin and testis) have clinicopathological features and outcomes different from conventional nodal FL [1]. The skin and gastrointestinal (GI) tract are the most commonly involved extranodal sites. The site of involvement may affect disease prognosis [3]. It is well recognized that stage I FL of the skin and duodenal-type FL have a significantly better outcome than nodal primary disease. Differently, stage I FLs of muscle, connective tissue and nervous system have significantly worse survival than nodal FLs. Other extranodal sites such as head and neck or respiratory system are not associated with worse survival.

Duodenal-type FL

Primary duodenal FL is a variant with distinctive biological and clinical features. It consists of polypoid lesions of the small bowel, more often in the second portion of duodenum [4]. Patients are usually asymptomatic. The disease is localized and incidentally detected. Classically, small nodules involve mucosa and submucosa. The cellular composition recapitulates low-grade FL, with centrocytes and only rare centroblasts (Fig. 1). The cells are positive for CD10, CD20, BCL6 and BCL2 and carry t(14;18)/IGH/BCL2 (Fig. 2). Primary duodenal FL has an indolent clinical course, often without therapy. Radiotherapy can be used. Local recurrences can occur. Clinical evaluation and staging is essential to exclude systemic FL secondarily involving the bowel, which follows a more aggressive course [5].

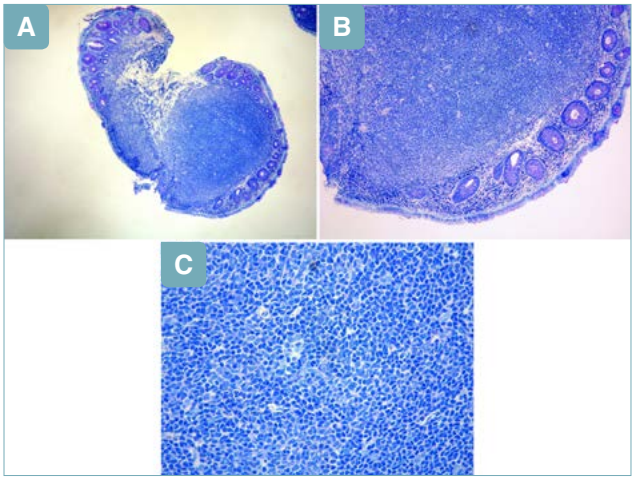

Figure 1. A Ileum. Follicular lymphoma, duodenal-type. Giemsa, 100x; B Ileum. Follicular lymphoma, duodenal-type. Giemsa, 200x; C Ileum. Follicular lymphoma, duodenal-type. Giemsa, 400x.

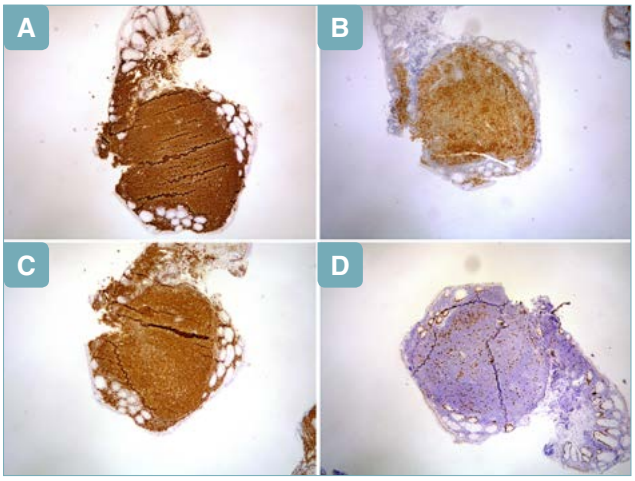

Figure 2. A Ileum. Follicular lymphoma, duodenal-type. CD20 immunostaining, 100x; B Ileum. Follicular lymphoma, duodenal-type. BCL6 immunostaining, 100x; C Ileum. Follicular lymphoma, duodenal-type. BCL2 immunostaining, 100x; D Ileum. Follicular lymphoma, duodenal-type. KI67/MIB1 immunostaining, 100x.

In this setting, multiple lymphomatous polyposis (MLP) needs to be mentioned, which is a rare clinical presentation of primary gastrointestinal lymphomas. MLP patients typically present multiple polypoid lesions of different sizes throughout the gastrointestinal tract. Most cases are mantle cell lymphoma (MCL), others originate from mucosa-associated lymphoid tissue (MALT lymphoma) and a few cases are FLs [6-8].

Testicular FL

Primary testicular FL was initially described in children and subsequently in adults (Fig. 3) [9,10]. It is usually a low stage disease (stage 1E). Histologically, it tends to show high-grade morphology (grade 3A) and the pattern of growth can be follicular or follicular and diffuse. Prominent fibrosis is often present. (Fig. 4). Testicular FL expresses germinal center (GC) markers (CD10, BCL6), but it usually lacks BCL2-protein expression and BCL2 gene rearrangements (Fig. 5). FL of the testis is typically a localized disease with an indolent clinical behavior and good prognosis. Optimal therapy for patients with low stage disease is not well defined. Most patients (children and adults) are treated with surgery plus anthracycline-containing chemotherapy, sometimes with central nervous system prophylaxis. In adults, primary FL of the testis needs to be distinguished from diffuse large B-cell lymphoma (DLBCL)

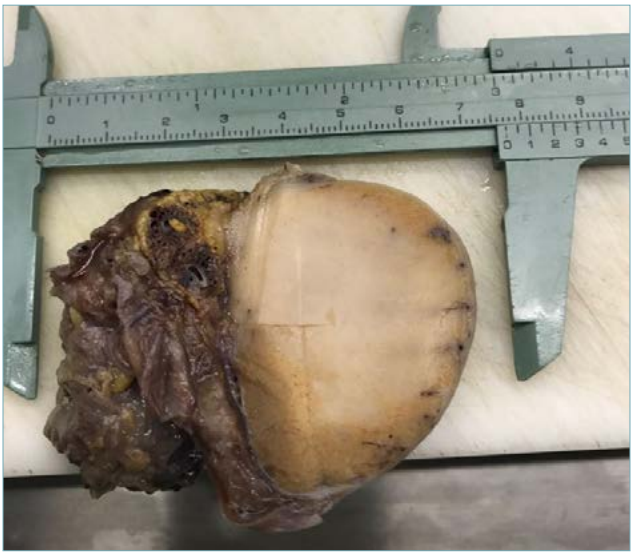

Figure 3. Testis. Primary follicular lymphoma of the testis. Gross appearance. Courtesy of Dr. A. Pireddu, Anatomia Patologica, Ospedale di Città di Castello, Perugia, Italy.

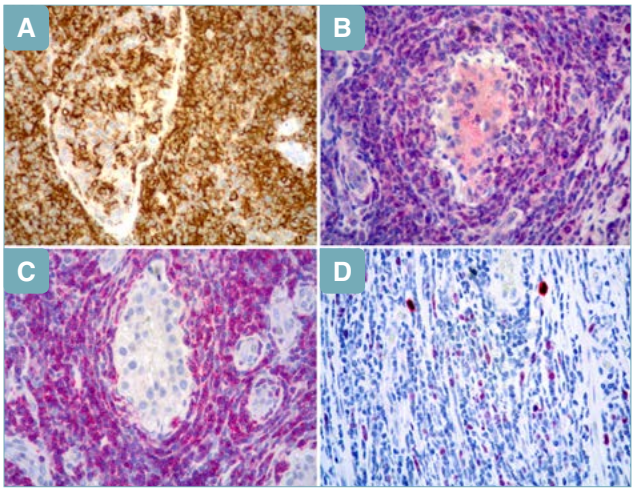

Figure 5. A Follicular lymphoma of the testis. CD10 immunostaining, 400x; B Follicular lymphoma of the testis. BCL6 immunostaining, 400x; C Follicular lymphoma of the testis. BCL2 immunostaining, 400x; D Follicular lymphoma of the testis. Ki-67/MIB1 immunostaining, 400x.

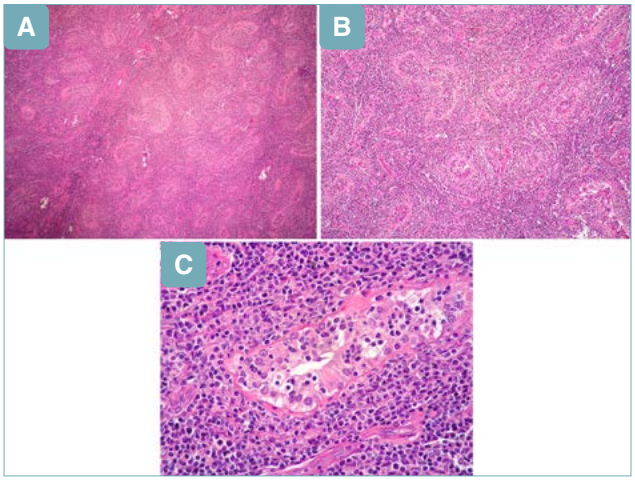

Figure 4. A Follicular lymphoma of the testis. HE, 100x; B Follicular lymphoma of the testis. HE, 200x; C Follicular lymphoma of the testis. HE, 400x.

(more commonly seen in the adult testis), which is a much more aggressive disease.

FL confined to the ovaries

FL arising primarily in the ovary is very rare and its clinicopathological features are not completely clear. Oznan et al. identified two main groups of FL arising in the ovary with divergent clinicopathological aspects [11]. The first group included cases with high-grade histology (3A), negativity or weak positivity for BCL2 and absence of BCL2 translocation; this group frequently had low stage disease. The second group included cases with low histological grade, strong positivity for BCL2 protein and presence of IGH/BCL2 translocation; this group frequently had advanced stage disease. There is no clear evidence that the second group arises primarily in the ovary, as the disease is usually in an advanced stage. The first group (high-grade, low stage) includes cases with disease usually confined to the ovary. This latter group may represent true primary ovarian FL. The features of high-grade, low stage BCL2-negative FL resemble those reported in FL of the testis, which shows a favorable outcome. It is tempting to speculate that this group of ovarian FL might be related to either pediatric-type FL (PTFL) or testicular FL [11].

Primary cutaneous FL

Primary cutaneous FL (PCFL) is a low-grade lymphoma of follicle center B cells, without evidence of systemic/nodal involvement at time of diagnosis. It is the most common primary cutaneous B-cell lymphoma. It presents with localized plaques or nodules on the scalp, trunk and back and rarely on the legs; multifocal skin lesions can be present. PCFL involves the dermis, often extending into subcutis. The epidermis is spared with a grenz zone separating the epidermis from the

underlying lymphoid proliferation (Fig. 6). Follicular and diffuse or totally diffuse pattern of growth may be present (Fig. 7) [12]. Neoplastic follicles are usually closely packed, irregular and not polarized, tingible body macrophages are absent, and mantle zone is thin or absent. Neoplastic cells include centrocytes and centroblasts. Rarely, centrocytes may be spindle-shaped or show bizarre features with different sizes and shapes [12]. Sclerosis and myxoid features may also be present in PCFL, particularly in the spindle cell variant [12]. Reactive T cells can be prominent. PCFL with a diffuse pattern of growth is entirely composed of sheets of centrocytes and centroblasts. The neoplasm may show any grade, although high-grade cytology (grade 3) is seen quite frequently and differential diagnosis with DLBCL legtype is mandatory. BCL6 is positive; CD10 is positive in cases with a follicular growth pattern, whereas it is often negative in cases with predominantly diffuse pattern (Fig. 8) [13]. Although BCL2 is often reported as negative or weakly positive and t(14;18)/IGH-BCL2 is frequently absent, recent studies have identified BCL2 expression as well as the presence of BCL2 rearrangements in a proportion of PCFL [14,15]. IRF4/MUM1 and FOXP1 are usually absent. Ki-67 shows a low proliferation index. Some PCFLs, mainly those composed of large cells and which need to be distinguished from DLBCL legtype, can show high Ki-67. PCFL patients usually have an indolent disease, regardless of grade. Unlike nodal FL, PCFL should not be graded histologically, because grading does not seem to provide prognostic information. Surgical excision and local radiotherapy are treatments of choice for localized disease. Rare cases with multifocal skin lesions and extensive cutaneous disease require systemic therapy. Local recurrences are reported (20-30% of cases). When extracutaneous sites are involved, regional lymph nodes and bone marrow are usually affected [12]. Transformation to DLBCL has been suggested by some studies [16].

Systemic FL secondarily involving the skin needs to be excluded. This scenario is challenging for pathologists, because careful clinical workup and staging are usually unavailable at time of skin biopsy. PCFL with diffuse pattern and numerous large centrocytes and centroblasts can be tricky to separate from DL-

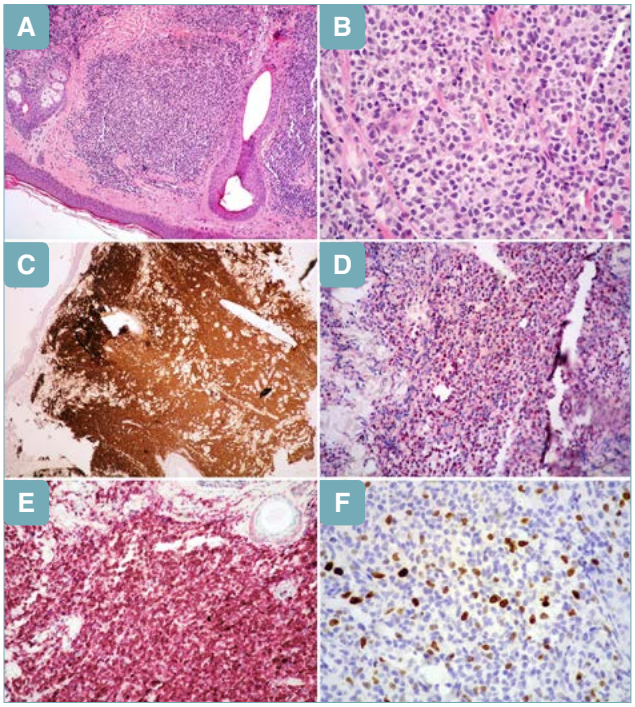

Figure 6. A Primary cutaneous follicular lymphoma. HE, 200x; B Primary cutaneous follicular lymphoma. HE, 400x; C Primary cutaneous follicular lymphoma. CD20 immunostaining, 100x; D Primary cutaneous follicular lymphoma. BCL6 immunostaining, 100x; E Primary cutaneous follicular lymphoma. BCL2 immunostaining, 100x; F Primary cutaneous follicular lymphoma. Ki-67/MIB1 immunostaining, 400x.

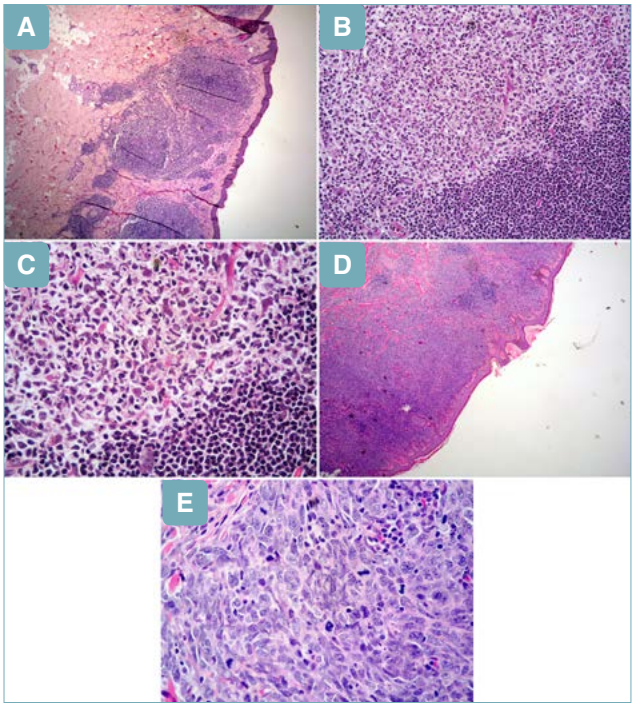

Figure 7. A Primary cutaneous follicular lymphoma, follicular pattern of growth. HE, 100x; B Primary cutaneous follicular lymphoma, follicular pattern of growth. HE, 200x; C Primary cutaneous follicular lymphoma, follicular pattern of growth. HE, 400x; D Primary cutaneous follicular lymphoma, diffuse pattern of growth. HE, 40x; E Primary cutaneous follicular lymphoma, diffuse pattern of growth. Large-sized neoplastic cells. HE, 400x.

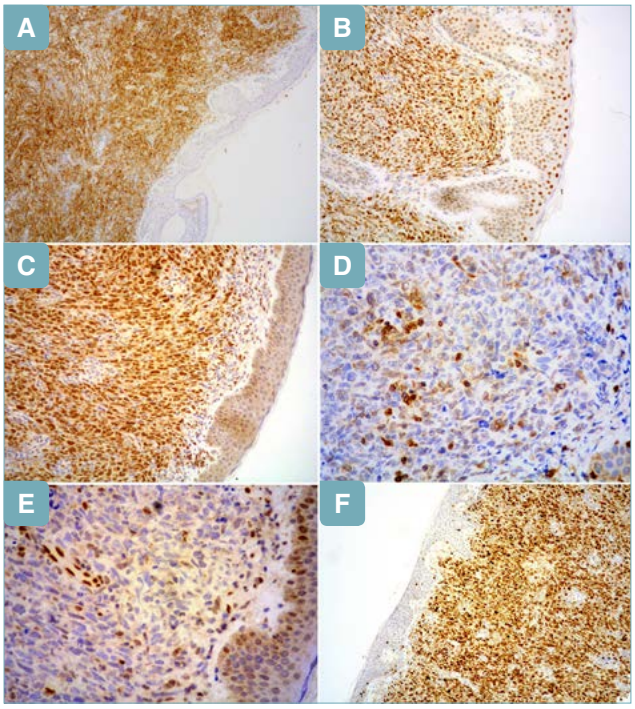

Figure 8. A Primary cutaneous follicular lymphoma, diffuse pattern of growth. CD10 immunostaining, 100x; B Primary cutaneous follicular lymphoma, diffuse pattern of growth. Large-sized neoplastic cells. BCL6 immunostaining, 200x; C Primary cutaneous follicular lymphoma, diffuse pattern of growth. Large-sized neoplastic cells. LMO2 immunostaining, 200x; D Primary cutaneous follicular lymphoma, diffuse pattern of growth. Large-sized neoplastic cells. BCL2 immunostaining, 400x; E Primary cutaneous follicular lymphoma, diffuse pattern of growth. Large-sized neoplastic cells. FOXP1 immunostaining, 400x; F Primary cutaneous follicular lymphoma, diffuse pattern of growth. Large-sized neoplastic cells. KI-67/MIB1 immunostaining, 200x.

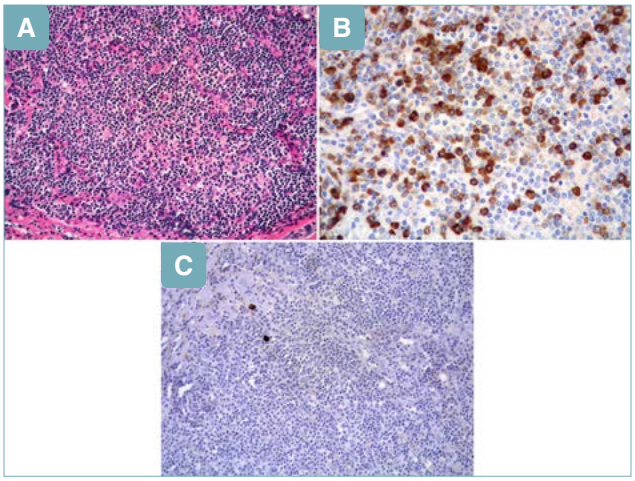

Figure 9. A Skin. Marginal lymphoma. Small, medium-sized lymphoid cells and few mature plasma cells. HE, 200x; B Skin. Marginal lymphoma. Monotypic mature plasma cell component. Kappa immunostaining, 200x; C Skin. Marginal lymphoma. Monotypic mature plasma cell component. Lambda immunostaining, 200x.

BCL leg-type. The presence of centrocytes, follicular dendritic cells meshwork and numerous T cells argue in favor of PCFL. Sheets of large atypical centroblasts and/or immunoblasts support DLBCL leg-type. MUM1 and FOXP1 are usually absent in PCFL, differently from DLBCL leg-type [12].

Primary cutaneous marginal zone B-cell lymphoma (PCMZBCL) needs to be distinguished from PCFL (Fig. 9). It shows a nodular or diffuse infiltrate within the dermis and sometimes subcutis, with sparing of epidermis. At low power, PCMZBCL typically shows nodular infiltrates containing reactive GC surrounded by small- and medium-sized cells with pale cytoplasm (marginal-zone, centrocyte-like cells). Characteristically, PCMZBCL has a more heterogeneous cytological appearance, including small- to medium-sized mature centrocyte-like lymphocytes, monocytoid lymphocytes with abundant clear cytoplasm and plasma cells. Dutcher bodies may be identified. PCMZBCL lacks expression of BCL6, CD10, and CD5. Light chain restricted plasma cells are commonly found at the periphery of the lesion [17].

Primary cutaneous follicular helper T-cell lymphoma (PCFHTCL) is a relatively recently described lymphoma mimicking PCFL. Patients tend to be elderly, presenting with multiple nodules, papules and/or plaques, often involving the extremities [18]. PCFHTCL displays a nodular architecture, syringotropism and includes numerous B cells. A careful examination reveals atypical T-cells with a follicular T-helper phenotype, including CD10 and BCL6 [19]. Systemic nodal follicular T-cell lymphoma may secondarily involve the skin; it can mimic follicular B-cell lymphoma.

Blastic plasmacytoid dendritic cell neoplasm is a rare myeloid neoplasm of immature plasmacytoid dendritic cells, commonly involving the skin. It can have a leukemic presentation or it can be limited to the skin, at least initially (Fig. 10). It follows an aggressive course, even in patients with skin-limited lesions. It may show a nodular growth pattern and include centrocyte-like cells. The expression of CD123, CD56, CD4, TCL1, CD2AP and CD303 helps with its correct classification [20].

Cutaneous reactive lymphoid hyperplasia may also mimic PCFL. In reactive hyperplasia, follicles show the typical outer mantle zone, encircling well polarized GC,

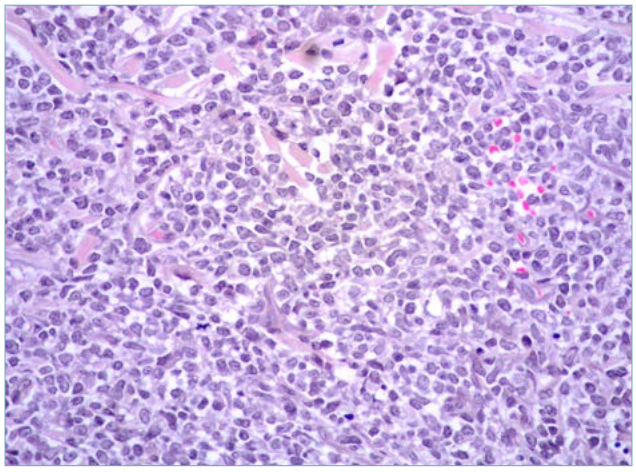

Figure 10. Skin. Blastic plasmacytoid dendriitic cell neoplasm. "Centrocyte-like" features of the neoplastic elements. HE, 400x.

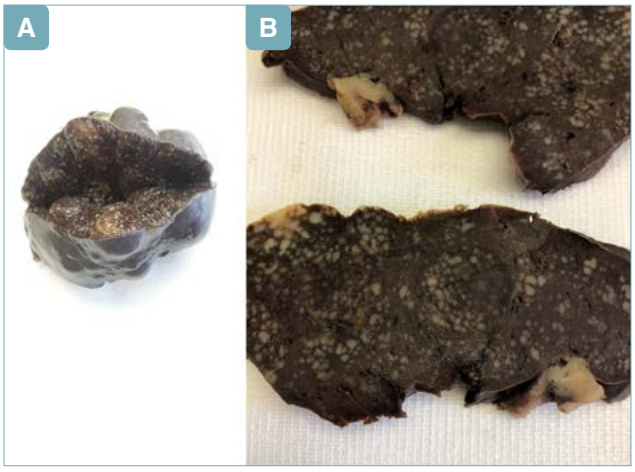

Figure 11. A Follicular lymphoma of the spleen, gross appearance. Courtesy of Dr. Marco Toraldo, Anatomia Patologica, Ospedale di Foligno; B Follicular lymphoma of the spleen, gross appearance. Courtesy of Dr. Marco Toraldo, Anatomia Patologica, Ospedale di Foligno.

as highlighted by Ki-67. Molecular studies (polymerase chain reaction - PCR) can help support PCFL diagnosis, examining immunoglobulin heavy-chain and light-chain genes and confirming clonality. The presence of clonality supports PCFL, if in the right clinicopathological context. However, pitfalls do exist. DNA may be of insufficient quality for molecular analysis, particularly in formalin-fixed, paraffin-embedded tissue. When DNA is of poor quality, false-negative results can occur [21]. PCFL may not have a detectable monoclonal population and false-negative results are possible [22]. In addition, when a B-cell population emerges as dominant clone in reactive lymphoid hyperplasia, false-positive results can occur [23].

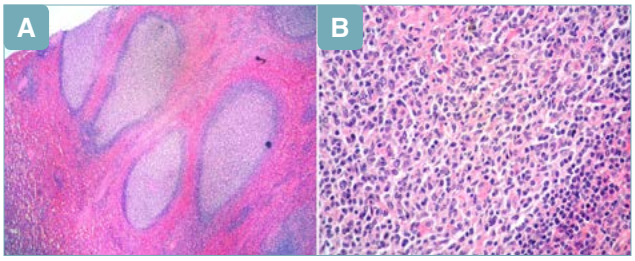

Figure 12. A Follicular lymphoma of the spleen, follicular pattern of growth. HE, 100x; B Follicular lymphoma of the spleen. Numerous centrocytes and few centroblasts. HE, 400x.

Splenic FL

Primary splenic FL is very rare. To date, just a few studies on primary splenic FL have been performed [24]. Its macroscopic appearance with multiple, small nodules can resemble splenic marginal zone lymphoma (Fig. 11). Histologically, the spleen shows a micronodular pattern, GC cytology and frequent marginal zone-like cells at nodules periphery (Fig. 12). Mollejo et al. reported the clinicopathological features of primary splenic FL subdividing it in 2 groups: the first resembling classical FL with the presence of t(14;18) and CD10 expression, which is usually diagnosed at advanced stage; the second, characterized by high grading, elevated proliferation index and BCL2 negativity, more often restricted to the spleen (Fig. 13) [24]. Splenic FL shows some clinical features different from nodal FL. Hepatitis C virus (HCV)-positive status is significantly more common in patients with splenic FL. Ann Arbor stage III or IV and high-risk FLIPI (Follicular Lymphoma International Prognostic Index) are less common in splenic FL. The progression-free survival is worse in patients undergoing splenectomy without postoperative chemotherapy. These results suggest the spleen itself, as primary lesion, might affect the biological characteristics of FL. Splenic FL should probably be considered a distinct type of FL compared to nodal FL [25].

Rare extranodal sites involved by FL

Rarely, FL may involve uncommon extranodal sites such as peripheral nerves, muscle, peritoneum, dura, pancreas, conjunctiva and orbit [26-33].

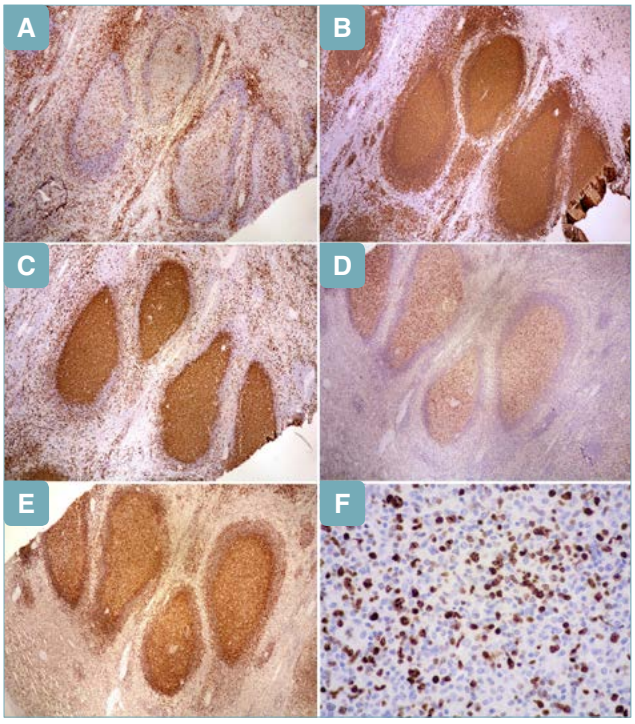

Figure 13. A Follicular lymphoma of the spleen. CD3-negative neoplastic elements, 100x; B Follicular lymphoma of the spleen. CD20-positive neoplastic elements, 100x; C Follicular lymphoma of the spleen. CD10-positive neoplastic elements, 100x; D Follicular lymphoma of the spleen. BCL6-positive neoplastic elements, 100x; E Follicular lymphoma of the spleen. BCL2-positive neoplastic elements, 100x; F Follicular lymphoma of the spleen. Proliferative index of neoplastic elements (KI-67/MIB1 15-20%), 100x.

Unusual phenotypes and molecular pitfalls in FL

BCL2-negative FL. BCL2 protein expression varies from 85-90% in grade 1-2 to less than 50% in grade 3 FL. BCL2 expression is related to the recurrent translocation t(14,18)(q32;q21) involving IGH and BCL2. BCL2-negative FL are explained by either true absence of t(14;18) or by mutation in BCL2 epitope usually recognized by clone 124 anti-BCL2 antibody. In these cases of BCL2 "pseudo-negative" FL, neoplastic follicles are immunoreactive using different anti-BCL2 antibodies such as clones E17 and/or SP66 [34,35]. Thus, the absence of BCL2 should not be interpreted as evidence against FL diagnosis, if other features are consistent with FL. Furthermore, the use of additional clones of anti-BCL2 antibody in the work-up of BCL2-negative FL is advisable.

CD10/BCL6 negative FL. A subset of FL, more frequently grade 3A, is CD10-negative and/or BCL6-negative. Recently, novel markers like Stathmin, GCET1, HGAL, and LMO2 have been introduced that can be useful in CD10 and/or BCL6-negative FL [36].

CD30 positive FL. A small percentage of FL, mostly grade 3, may contain sparse CD30-positive cells. This phenomenon is usually restricted to large centroblasts and/or to pleomorphic Hodgkin-Reed Sternberg (HRS)-like cells of grade 3 FL [37].

CD5 positive FL. CD5 is expressed by 5% of FL. CD5-positive FL can have the floral and/or diffuse patterns of growth. CD5 expression has been associated with higher International Prognostic Index (IPI), higher rate of transformation, and shorter progression-free survival [38].

IRF4/MUM1 positive FL. IRF4/MUM1 expression is detected in grade 3B FL as marker of late GC differentiation [39]. Low to moderate IRF4/MUM1 expression may be observed even in low-grade FL. High IRF4/MUM1 expression has been recently reported to be predictive of poor outcome in low-grade FL [40].

The t(14;18)(q32;q21)/IGH-BCL2 translocation is present in common FL, although its frequency varies greatly depending on FL grading. The translocation t(14;18) is detected in up to 90% of low-grade FL, but in only 60-70% of grade 3A and 15-30% of grade 3B FL [41]. Furthermore, BCL2 translocation variants such as t(2;18) and t(18;22) have been described. FL with BCL6 translocation represents 10-15% of cases, more frequently grade 3A and 3B. These FL strongly express BCL6, but are quite often BCL2 and/or CD10 negative [42].

The updated WHO classification recognizes the category of high-grade B-cell lymphoma (HGBCL) with MYC and BCL2 and/or BCL6 rearrangements, so called double-hit (DH) or triple-hit (TH) lymphomas [1]. Occasionally, "de novo" low-grade or grade 3 FL may carry MYC and BCL2 and/or BCL6 gene rearrangements, but should not be classified as HGBCL, unless undergoing a clear-cut transformation. The prognostic significance of concurrent MYC and BCL2 or BCL6 rearrangements in otherwise typical FL is an open question. Some studies report an aggressive course, but better response to more intensive regimens, while others show a behavior similar to FL lacking MYC rearrangement [43-45].

Elucidation of the clinicopathological features of NOTCH-mutant FL. NOTCH1 and NOTCH2 have been recently reported in several B cell lymphoma. The role of these mutations in FL is not known. A recent study identified NOTCH1 and NOTCH2 mutations in 6.3% of FL. NOTCH-mutated FL showed lower frequency of t(14;18), higher incidence of splenic involvement and female predominance. Furthermore, transforma-

tion was more frequently identified in NOTCH-mutated FL than in wild-type cases. These results indicate NOTCH mutations are uncommon in FL, but may occur in a subset of cases with distinctive features [46].

Grade 3B FL

The number of centroblasts is the key feature for FL grading. Grade 3B is a FL with a purely follicular growth pattern, composed only by centroblasts (Fig. 14). Pure 3B FL is rare and clinical data on this enigmatic entity remain scarce [39]. Most 3B FLs focally contain diffuse areas, therefore, deserving the diagnosis of DLBCL [47]. Furthermore, grade 3B rarely coexists with grade 1-2 or 3A, suggesting a divergent pathogenesis [48]. Grade 3B FL is generally CD10-negative and IRF4/MUM1-positive (Fig. 15). The translocation t(14;18)(q32;q21) juxtaposing the IGH and BCL2 genes is rare in pure 3B FL (13%), despite expressing BCL2 protein in 69% of cases. BCL6 rearrangement occurs rarely in pure 3B FL, whereas increased TP53 expression is rather common (31%). Grade 3B FL is still an evolving subclass. Physicians should understand its aggressive nature, requiring timely attention, compared with grade 3A. In many aspects 3B FL resembles de novo DLBCL. Some studies suggest it may represent a morphological variant of DLBCL with a follicular pattern of growth [39]. Histology, immunophenotypic profile and chromosomal aberrations of pure 3B FL resemble DLBCL, particularly non-GCB type. It is widely accepted 3B FL is distinct from other types of FL and it is intriguing to speculate it may represent a follicular growing variant of DLBCL.

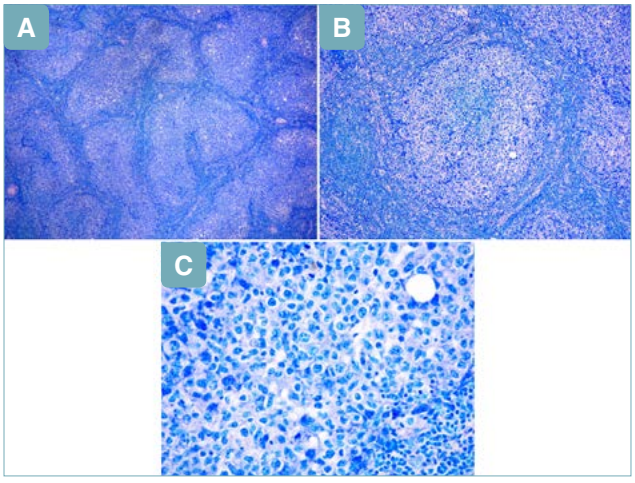

Figure 14. A Lymph node. Follicular lymphoma 3B. Giemsa, 40x; B Lymph node. Follicular lymphoma 3B. Giemsa, 100x; C Lymph node. Follicular lymphoma 3B. Giemsa, 400x.

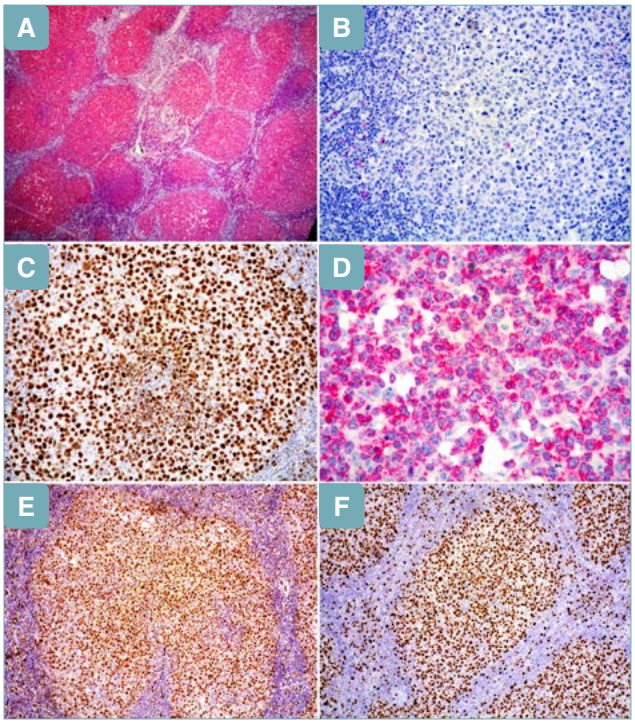

Figure 15. A Lymph node. Follicular lymphoma 3B. CD20 immunostaining, 40x; B Lymph node. Follicular lymphoma 3B. CD10 immunostaining, 200x; C Lymph node. Follicular lymphoma 3B. BCL6 immunostaining, 200x; D Lymph node. Follicular lymphoma 3B. BCL2 immunostaining, 400x; E Lymph node. Follicular lymphoma 3B. LMO2 immunostaining, 100x; F Lymph node. Follicular lymphoma 3B. KI-67/MIB1 immunostaining, 100x.

Transformation of nodal FL

It is well recognized that clinical aggressiveness and risk of transformation to DLBCL increase proportionally to the number of centroblasts and proliferative fraction. Transformation or progression occurs in 30% of FL. The current WHO criteria for transformed FL include a diffuse pattern of growth with centroblasts > 15/HPF (grade 3) [1]. In other words, the presence of grade 3 cytology in a diffuse pattern constitutes a DLBCL.

Currently, transformed FL is classified as DLBCL, or high-grade B-cell lymphoma (HGBCL) with MYC, BCL2 and/or BCL6 rearrangements or HGBCL not otherwise specified (in absence of MYC, BCL2 and/or BCL6 rearrangements). The HGBCL category has variable morphology, including DLBCL, Burkitt lymphoma, and/or "blastoid" morphology. "Burkitt-like" cases are reminiscent of both DLBCL and Burkitt lymphoma, not fulfilling diagnostic criteria for either entity.

Cases with so-called "blastoid morphology" show diffuse cohesive sheets of monotonous, small to medium-sized cells, high proliferation index, and starry-sky pattern. The cells have round nuclei with finely dispersed chromatin, inconspicuous nucleoli and a small rim of cytoplasm. These cells resemble lymphoblasts or the blastoid variant of MCL. Staining for TdT, Cyclin D1 and SOX11 should be performed.

More rarely, FL may transform into Hodgkin lymphoma, plasmablastic lymphoma, histiocytic sarcoma (HS) or precursor B cell lymphoblastic leukemia/lymphoma [49-52].

Lymphoblastic-type transformation of FL is a rare event with a poor outcome. It has to be differentiated from "de novo" FL with blastoid features, which has the typical FL phenotype and genetic abnormalities, without TdT expression. To avoid any confusion, the current WHO classification recommends the term "transformed FL of lymphoblastic type, TdT positive".

Recent studies suggested that transformation might occur in early neoplastic progenitors rather than in later subclones [53]. Thus, the phenomenon of transformation could be explained by a divergent evolution from a common precursor which was the founder cell of initial FL, and then evolved into DLBCL, histiocyte sarcoma, or lymphoblastic B-cell neoplasia [54].

Clonality studies are useful; matching rearrangements between initial FL and subsequent high grade lymphoma confirm a clonal relationship.

Composite FL

Composite lymphoma (CL) represents a fascinating process. It consists of two or more morphologically and immunophenotypically distinct lymphomas within the same anatomic site [55,56]. Its incidence ranges from 1 to 4.7% of total lymphomas, although CL may be more common than previously thought [57,58]. CL can arise synchronously or metachronously and can be clonally related or not. Regardless of the distinctive histology of the different components, in some cases the components are clonally related, whereas in others they are clonally unrelated, representing the "collision" of clonally unrelated tumors. With the advent of molecular analysis, it became clear that, in a subset of cases, CL components share a common clonal origin, suggesting derivation from a common precursor cell [59,60].

An adequate sampling is required to establish CL diagnosis. CL can be composed of FL and MCL, FL and chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), FL and nodal marginal zone lymphoma (NMZL), FL and nodular lymphocyte predominance Hodgkin lymphoma (NLPHL), FL and classic Hodgkin lymphoma (CHL). Cases of FL associated with DLBCL, HGBCL, Burkitt lymphoma and B-lymphoblastic lymphoma/leukemia have to be excluded, because they represent high-grade transformation.

CL with FL and MCL have been rarely reported. FL component is typically low-grade, BCL2 positive and harbors the t(14;18) translocation. The MCL component shows a diffuse or in situ mantle-zone growth pattern, it is CCND1 positive and harbors the t(11;14) translocation [61]. Morphologically, the nodal architecture is intact and reactive follicles are mainly distributed in the cortex. The mantle zone is preserved and CCND1 positive cells are often restricted to the mantle zone [62]. Some studies suggest FL and MCL are clonally related, originating from the same preneoplastic clone [59,63,64].

CL with FL and CLL/SLL is extremely rare (Fig. 16) [65]. An interesting study of Boiocchi et al. supported the notion that composite low-grade B-cell lymphomas are usually biclonal [66]. Another recent study, reporting the largest case series of composite CLL/SLL and FL did not perform microdissection, so that the relationship between the two components cannot be definitively determined [65].

Recent examples of composite FL and NMZL, studied by PCR sequencing of IGH from microdissected NMZL and FL components, showed different sequences in the CDR3 region, suggesting the presence of two different clones [67].

Rarely, composite FL and NLPHL have been described, but the clonal relationship was not established, due to insufficient tissue for laser capture microdissection [68,69]. Composite CHL and FL have been reported, in some of which the CHL and FL components were clonally related. CHL may display the translocation t(14;18), suggesting a common origin (common B-cell precursor) of CHL and FL components. In a series of 19 composite cases involving CHL and other non-Hodgkin lymphomas, a shared clonality was demonstrated in 12/19 (63%) cases [58,70,71].

T-cell lymphoma associated with low-grade B-cell lymphoma is very rare. A composite FL and T-cell lymphoma is rarely reported [72,73]. The genomic aberration may have occurred in an early lymphoid progenitor which underwent divergent evolution via additional genomic alterations, resulting in heterogeneous subclones and eventually T-cell and B-cell neoplasms [72].

Occasionally, small innocuous aggregates of Langherans cells are identified within FL. Histiocytic and Langherans cell neoplasms occurring synchronously or sequentially in FL patients have been reported. FL and Langherans cell neoplasms or histiocytic sarcoma often share a common cell precursor (clonally related) [51,74].

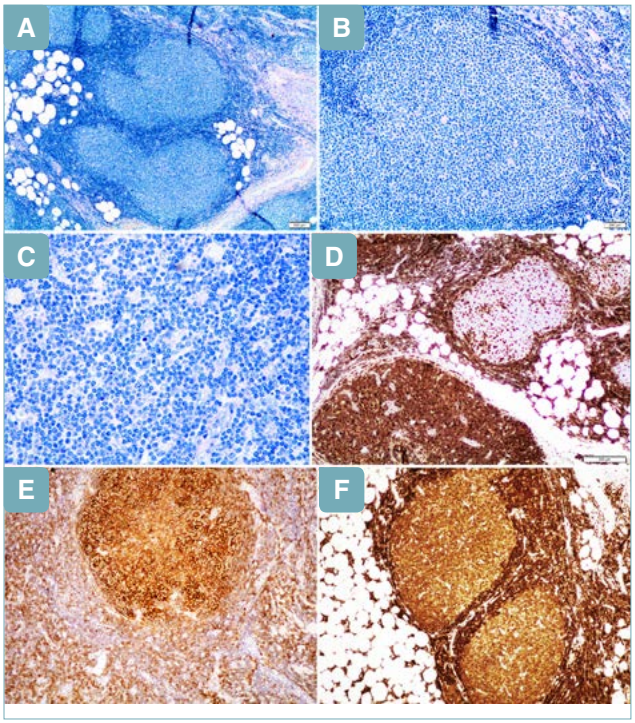

Figure 16. A Lymph node. Composite lymphoma: Follicular lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukaemia. Giemsa, 100x; B Lymph node. Composite lymphoma: Follicular lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukaemia. Giemsa, 200x; C Lymph node. Composite lymphoma: Follicular lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukaemia. Giemsa, 400x; D Lymph node. Composite lymphoma: Follicular lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukaemia. CD5 immunostaining. 100x; E Lymph node. Composite lymphoma: Follicular lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukaemia. CD23 immunostaining. 100x; F Lymph node. Composite lymphoma: Follicular lymphoma and small lymphocytic lymphoma/chronic lymphocytic leukaemia. BCL2 immunostaining. 100x.

CL diagnosis is challenging, requiring careful interpretation of morphology, immunohistochemistry and fluorescence in situ hybridization (FISH) analysis as well as flow cytometry, particularly when both components show identical immunoglobulin light chain restrictions and/or overlapping immunophenotypic features. A handful of CL have been reported, few of which have been characterized in terms of clonal relationships. IGH gene rearrangement analysis is critical to demonstrate the clonal relationship. It is recommended to use not only morphology, immunohistochemistry and FISH, but also PCR or next-generation sequencing (NGS) of the IGH and T-cell receptor gene rearrangements. Molecular studies are proving to be invaluable in CL workup. FISH, immunoglobulin rearrangement and sequencing as well as NGS technology can be improved by tissue microdissection. IGH analysis on whole tissue sections may not be helpful and laser capture microdissection is necessary to purify, or enrich individual components, in order to allow an interpretable gene rearrangement analysis. Thus, the power of microdissection coupled with molecular analysis needs to be considered. Recently, the concept is emerging that CL may represent different phenotypes of an identical shared common progenitor [71]. The analysis of additional CL cases is necessary to further investigate the clonal relationship between the individual components and to get better insights into CL pathogenesis.

Bone marrow involvement by FL

Usually, bone marrow trephine biopsy is performed for FL staging. Bone marrow involvement is quite common, occurring in 80% of FL patients. Typically, lymphoma is aligned along the trabecular bone (paratrabecular pattern), although interstitial and/or nodular patterns may be seen (Fig. 17). Rarely, bone marrow is extensively involved by FL (Fig. 18) [75]. Grading is not recommended on bone marrow biopsy. In absence of previous rituximab therapy, CD20 is sufficient to reveal even subtle bone marrow infiltration, whereas CD10

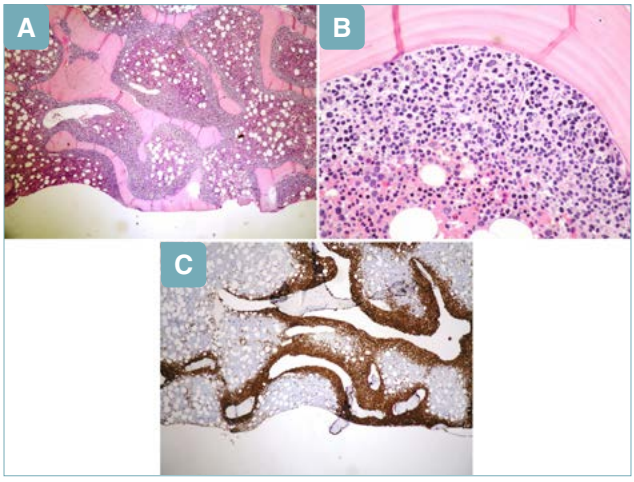

Figure 17. A Bone marrow. Follicular lymphoma, paratrabecular pattern of growth. HE, 100x; B Bone marrow. Follicular lymphoma, paratrabecular pattern of growth. HE, 400x; C Bone marrow. Follicular lymphoma, paratrabecular pattern of growth. CD20 immunostaining, 100x.

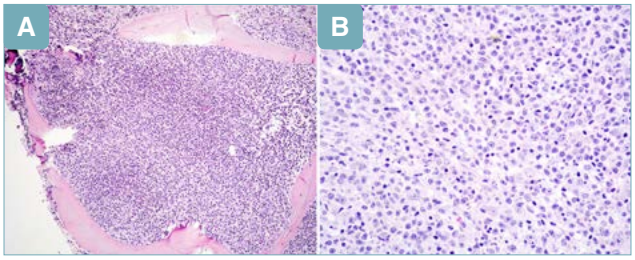

Figure 18. A Bone marrow. Follicular lymphoma, diffuse pattern of growth. HE, 100x; B Bone marrow. Follicular lymphoma, diffuse pattern of growth. HE, 200x.

and BCL6 are typically downregulated or may be totally negative. BCL2 staining is not useful, and does not add any further information. Furthermore, BCL2 is expressed by many other indolent low-grade B-cell lymphomas. Sometimes, a nodal transformed DLBCL coexists with low-grade FL in bone marrow, representing the so-called "discordant" lymphoma [76].

Approach to histopathological diagnosis of FL by core needle biopsy

Correct lymphoma classification is the best way to obtain relevant information for treatment and outcomes. The criteria for FL diagnosis and, by extension, the most appropriate therapeutic strategies are based largely on histologic evaluation of surgically excised specimens. Nonetheless, recently, an increasing reliance on core needle biopsy (CNB) of lymph nodes is evident. In many institutions, CNB is the primary diagnostic procedure in the suspect of lymphoma. Several studies on the effectiveness of CNB suggested that CNB yields an adequate diagnosis for treatment decision in about 65% to 75% of cases [77-80].

The reasons of the increasing popularity of this procedure are briefly summarized below. One of the most important considerations leading to CNB over excisional biopsy is urgency. However, in 25% of cases, CNB fails to yield an actionable diagnosis, further delaying therapy (Tab. I).

Since CNB can give only partial information, excisional biopsy of the lymph node should be performed, whenever possible. CNB has limitations, which is not particularly surprising, given how critical the architectural pattern is in FL diagnosis (Tab. II). Histological pattern, grading, immunohistochemical interpretation, including proliferative index, as well as detection of areas of transformation are common dilemmas, as many samples do not contain the recommended 10 follicles. Accurate grading may be very difficult on CNB. The National Comprehensive Cancer Network (NCCN) clinical practice guidelines in oncology are quite explicit, regarding the preference of excisional biopsies at the time of initial diagnosis, whenever feasible [77,80,81]. Despite the evolution of diagnostic methodologies, the use of ancillary techniques only occasionally compensates the loss of diagnostic specificity due to limited sampling.

The current WHO classification states "accurate grading cannot be performed on fine-needle aspiration and may be difficult on core needle biopsy. Therefore, an excisional biopsy is recommended for primary diagnosis."

Table I. Advantages of core needle biopsy.

1	Diagnostic urgency
2	Sparing surgical procedure to the patient [patient compliance]
3	Risk of complications minimized [little morbidity]
4	Lymph nodes difficult to be removed can be sampled [deep-seated lymph nodes]
5	75% less expensive than excisional lymph node biopsy [cost-effectiveness]
6	Excluding non-hematopoietic tumors
7	Staging patients with known lymphoma
8	Assessment for relapse of lymphoma [change in the histologic findings; transformation]

Table II. Disadvantages of core needle biopsy.

1	Small specimen size
2	Sampling error
3	Artifactual distortion of morphologic features
4	Adequacy of tissue for ancillary studies [exhaustion of the paraffin block]

Conclusion

Under the broad heading of FL, diseases with different clinicopathological features are included. Diverse molecular pathways are probably associated with different clinical features and outcomes.

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Case Report

Primary post-radiation angiosarcoma of the small bowel. Report of a case and review of the literature

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Summary
Angiosarcomas developing in unusual sites such as the small bowel are rare, and fewer than 65 cases have been reported in the literature. They are not uncommonly associated with a known eliciting factor. Thus, among hitherto described cases of angiosarcoma of the small bowel, 16 were radiation-induced. One additional example of ileal post-irradiation angiosarcoma (PRA) in a 72-year-old female patient with a past history of uterine leiomyosarcoma is herein reported as a reminder of this causal association. The morphologic and immunohistochemical clues leading to the correct diagnosis of PRA of the small bowel and the differential diagnostic problems are discussed; a comprehensive review of the literature has also been performed with a focus on survival.

Key words: angiosarcoma, small bowel, radiation

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Introduction

Postradiation sarcomas are uncommon malignant mesenchymal tumors with most reported cases diagnosed as malignant fibrous histiocytoma and less often as fibrosarcoma or osteosarcoma. Criteria to attribute a malignancy to be radiation induced are history of radiation, long latent period, histologically proven malignancy within the field of irradiation, and different histology of the new tumor if radiation was given for malignancy [1]. It is estimated that 50% of all cancer patients receive radiotherapy and, in particular, radiation is a proven efficacious treatment for breast carcinoma [1].
Most of the documented cases of postradiation angiosarcoma (PRA) are occurred in breast skin and underlying soft tissues including the chest wall. The remainder of the cases of PRA have been documented in various locations, but rarely occur in the gastrointestinal tract.
In this paper, we describe a case of PRA of the small bowel. We discuss the clinicopathological features and differential diagnosis, according to the previous literature review.

Case report

A 72-year-old woman was referred to the Emergency Department of

Hospital "San Giovanni di Dio", Crotone (Italy) because of intermittent abdominal pain and recent symptoms of intestinal obstruction. Review of medical history yielded positive results for uterine leiomyosarcoma diagnosed 24 years before presentation. At that time, after total hysterectomy, she had received external beam radiation (irradiation dose unknown) to the lower pelvis. Colonoscopy performed in March 2017 was unremarkable. An abdominal plain film was obtained, which showed dilated intestinal loops with transitions to nondilated ones, and intestinal tympanites.

At laparotomy, there were small bowel loops adhering to the pelvic floor, as well as a mass in the ileum at about 20 cm from ileocecal valve with dilatation of the small intestine proximal to it. The adhesions were sectioned and the bowel mobilized. Additionally, small intestine resections were performed at three different consecutive sites, one of them due to the severe bowel stricture, and the others due to risk of dehiscence of the first side to side isoperistaltic anastomosis and setting up of a new side to side antiperistaltic definitive anastomosis.

On gross examination, the longer piece corresponded to a 36 cm-long segment of small intestine revealing a narrowed tan central zone, measuring 2 cm in largest diameter, with superficial erosion. Light microscopy, at low-power field, showed a diffuse infiltrative prevalently submucosal growth of epithelioid to spindle cells with subtle clefting suggestive of vascular differentiation. Sheets and strands of epithelioid elements focally filled the lamina propria preserving the overlying glands and causing mucosal ulceration (Fig. 1). High-power features of the lesional cells included a scant amount of eosinophilic cytoplasm and a round to spindle nucleus with coarse chromatin, a sometimes prominent nucleolus, and cellular necrosis (Fig. 2). There were occasional intracytoplasmic lumina that contained red blood cells (Fig. 3). The mitotic activity was high with up to 29/10 high-power fields. Immunohistochemical studies showed diffuse expression of CD31 and vimentin and negative staining for AE1/AE3 and Cam 5.2 keratins, CD34, desmin, α-smooth-muscle actin, chromogranin, CD56, PAX-8, HHV-8, S-100 protein and h-caldesmon confirming the diagnosis of PRA. Furthermore, the tumor had a MIB-1 index of 80% (Fig. 4). Surgical margins of the specimen were viable and free of infiltration.

The patient was referred to Oncology center to receive adjuvant therapy. She was discharged in good general conditions at 6 days after surgery, and the follow-up of 26 months remained uneventful.

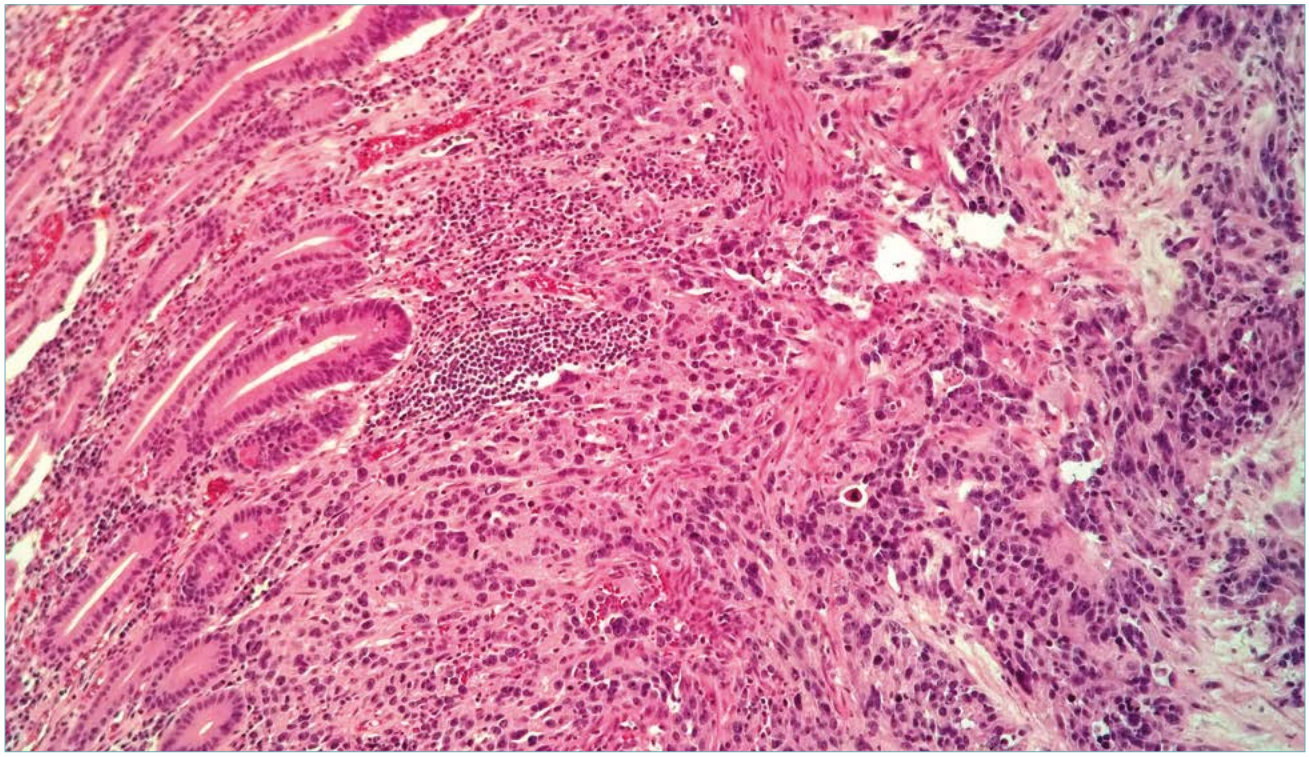

Figure 1. The tumor was located in the mucosa and submucosa layer and had a predominantly solid growth pattern.

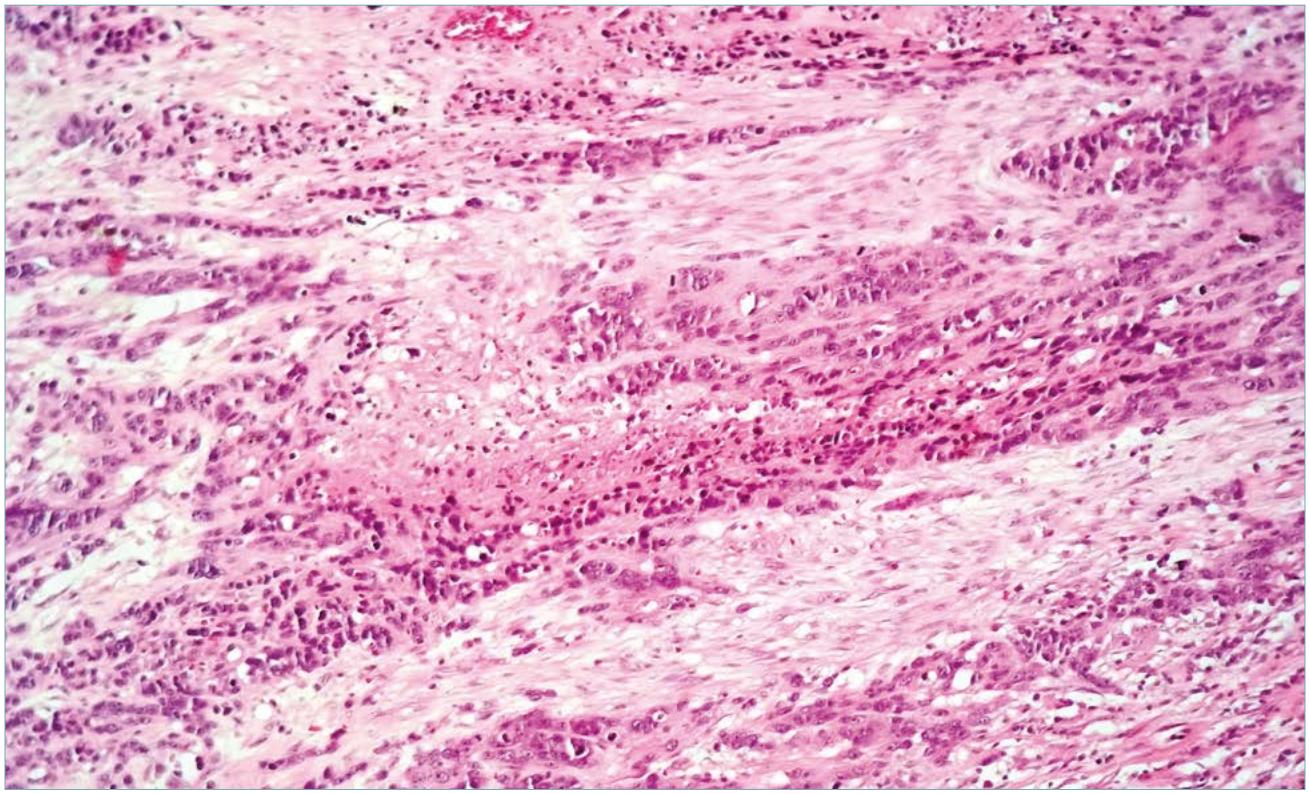

Figure 2. Sheets and cords of epithelioid to spindle neoplastic cells with eosinophilic cytoplasm and central necrotic zone.

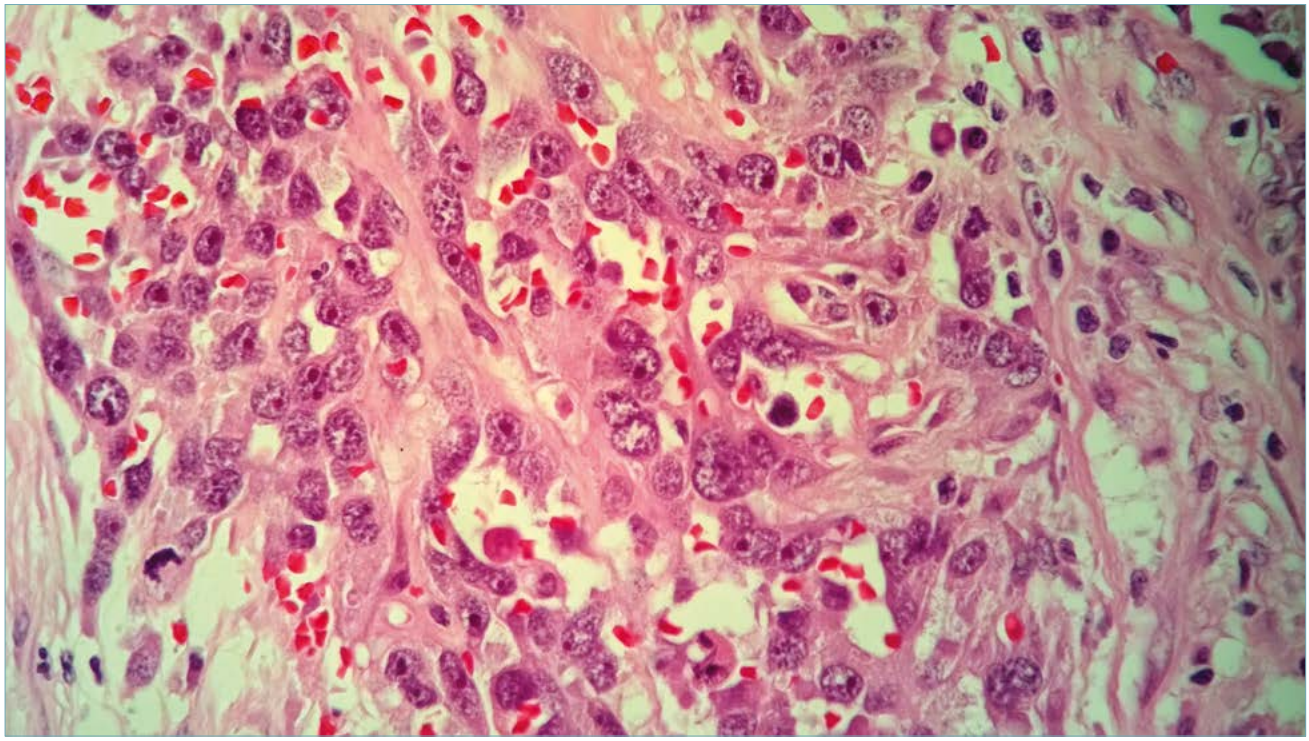

Figure 3. High-power view of the neoplasm displaying tumor cells with lightly eosinophilic cytoplasm, large vesicular nuclei and prominent nucleoli. There are subtle clefting and the presence of intracytoplasmic lumina containing red blood cells suggestive of vascular differentiation.

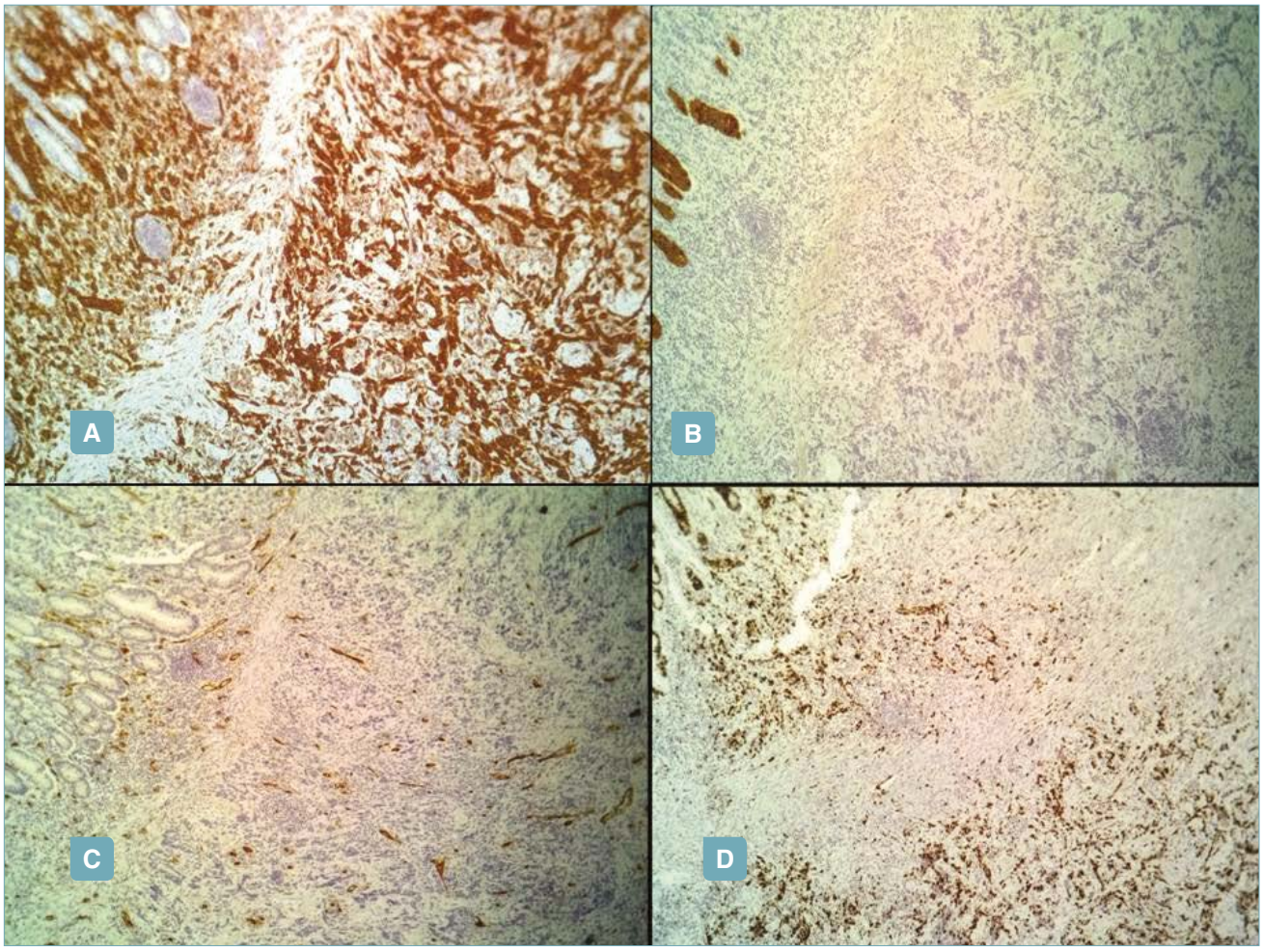

Figure 4. (A)The spindle to epithelioid tumor cells are strongly positive for CD31; (B) The neoplastic cells are negative for AE1/AE3 cytokeratin; (C) No staining of tumor cells for CD34; (D) High proliferative index of tumor cells with MIB1 immunostaining.

Discussion

Primary malignant tumors of the small bowel are unusual, comprisizing less than 2% of gastrointestinal tumors. Among these, the great majority are malignant carcinoids, adenocarcinomas and leiomyosarcomas, whereas other soft tissue tumors are very rare [2].
Angiosarcoma is a malignant tumor characterized by the hyperproliferation of cells with endothelial vascular features. These very rare neoplasms represent about 1-2% of all sarcomas and can occur anywhere in skin and subcutaneous tissues, particularly in the head and neck region. Angiosarcoma infrequently presents primarily with disease of internal organs, predominantly liver, spleen and adrenals [3].
In a review of 106 cases of gastrointestinal vascular tumors seen at the Mayo Clinic between 1925 and 1944, only 14 angiosarcomas were found, 3 of which in the small bowel [4]. Over the years, rare cases proposed to represent angiosarcoma have also been described in several reviews of primary malignancies of the small intestine, in scattered case reports and small series [5,6]. From 1949 to date, a Medline search using the key words “angiosarcoma” and “small bowel” revealed about 61 cases of primary angiosarcoma of the small bowel [1-52]. From published data, including the current case, the age of presentation ranged from 22 to 87 years (mean, 60.8 years) and there were 21 women (35.6%) and 38 males (64.4%), and two patients whose gender was not specified [1-52]. The male to female ratio was: 1,8:1. In our review of literature, the predominant presenting signs and symptoms for this rare entity were gastrointestinal bleeding, which occurred in 29 patients (49.1% of cases) and abdomi-

nal pain (44% of cases), often associated with anemia (28.8% of cases) [1-6,9,11-19,21-28,30-35,37-39,42,43,45-52]. In addition, these patients were often submitted to frequent blood transfusions, and death sometimes resulted from incontrollable hemorrhage. There were only eight cases that showed clinical evidence of small bowel obstruction [7,19,27,30,39]. Three patients had a history of initial perforation and acute abdomen, and the clinical picture of another case was thought to be consistent with an appendiceal abscess [5,22,31,36].

Because of the relative inaccessibility of the deep small bowel for endoscopic investigations and the lack of accuracy of radiographic features, the preoperative diagnosis of small bowel angiosarcoma remains unlikely. The conventional diagnostic methods, such as traditional endoscopy, barium studies, and even technetium 99m-labeled erythrocyte scan might leave the physician without an answer, and capsule endoscopy or MR enteroclysis might help detect the source of bleeding, only in some cases [3,14,18,21,27]. Briefly, in almost all patients reported in literature to date, including the current case, the correct diagnosis of angiosarcoma was made postoperatively after histologic and immunohistochemical examination of the surgical specimen. The association between angiosarcomas and certain environmental toxins or previous external-beam radiation therapy is well-documented. Occupational exposure to vinyl chloride, thorotrast and arsenic has been associated with its pathogenesis [2]. Furthermore, studies have clarified the relationship between angiosarcoma, chronic lymphedema of various etiologies and genetic factors, as neurofibromatosis NF-1, Klippel-Treunaney and Maffucci syndromes, mutated BRCA1 or BRCA2 [6,26]. To date, of the 61 reported cases, only 17 (27.8%) have been examples of PRA arising in the small bowel (including the case under discussion) [1,2,7,10,19,20,29,31,36-40,42]. Documented cases of PRA of the small bowel are listed in Table I. There was a significant difference in the incidence between male and female and most patients were women (male to female ratio:1:3). Patients were irradiated earlier for various cancers, including Hodgkin's lymphoma, chondrosarcoma, endometrial and cervical carcinoma, uterine leiomyosarcoma, and ovarian malignancies [1,7,10,19,20,29,31,36-40]. The rest of them were two male subjects, one with a history of antecedent radiotherapy for unknown pelvic tumor and the other with a prolonged exposure to both occupational radiation and polivinyl chloride [2,42]. Additionally, considering the long-term involvement of two patients in the construction industry, they might have also been exposed to chemicals implicated in the cause of this disease [23,49]. Apart from radiation and chemicals, angiosarcomas are also induced by foreign bodies [50]. Though there are many cases of foreign body associated sarcomas of various sites, it is rare in the intestinal tract possibly due to fewer chances of harboring foreign bodies at this site [2,50]. The interval between radiation and presentation of PRA ranged from 3 to 24 years (mean, 11 years) [1,7,10,19,20,29,31,36-40,42]. The terminal ileum was the most common location of PRA reported in the small bowel (at least 8 of the 17 cases) [1,7,20,31,36,37,40]. In two patients the neoplasm spread locally to involve the large bowel [39]. Approximately 45-50% of patients presented with advanced local disease due to multifocal intestinal involvement and early widespread metastasis to intra-abdominal and pelvic structures, including the peritoneal surface [2,7,10,20,29,31,42]. Patients who survived for longer periods developed intractable local disease and distant metastasis to liver, spleen, lungs and pleura [1,10,19,31,36,37,39,40]. Overall, 15/17 of patients had follow-up information available [1,2,7,10,19,29,31,36-40]. Follow-up ranged from 14 days to 36 months, mean = 11 months [1,2,7,10,19,29,31,36-40]. Of the 15 patients, all were died of disease at variable time points – from 14 days to 36 months – except two, one which was alive with disease at 21 months and the other, our patient, which had no signs of recurrence at 26 months. Prognostically, PRAs resemble other small bowel angiosarcomas (follow-up data: from 9 days to 48 months, mean = 10.4 months, average survival time of about 30 weeks) including poor response to combined chemotherapy regimens [4-6,8,9,11,12,15,17,18,23-26,28,32-34,41,43,44,46-49]. Therefore, the aggressive biologic behavior of PRA of small bowel is of no surprise.

Macroscopic findings of PRA of the small intestine frequently include a markedly hemorrhagic appearance with poorly defined thickening of the bowel wall and/or black nodules scattered on the serosal surface. Microscopically, our case was similar to the previously reported ones. The histopathologic evaluation of PRA, similarly to non-radiation-induced angiosarcoma arising in superficial soft tissue and gastrointestinal tract, can be a challenge, as it can exhibit histologic differentiation varying from benign appearing vascular proliferation to undifferentiated malignancy, with slit-like vascular channels and sheets of spindle to epithelioid neoplastic cells with or without intraluminal red blood cells, as the sole evidence of vascular differentiation. The presence of a lobular architecture and/or a papillary growth pattern, particularly when localized, are a histologic presentation that includes rare benign vascular proliferations occurring in the gastrointestinal tract related to intussusception and mucosal prolapse and the intravascular papillary endothelial hyperplasia. These lesions show minimal nuclear atypia and low mitotic rate [53].

Other differential diagnostic considerations include

Table I. Reported cases of PRA of the small bowel.

Reference	Sex/ age (years)	Presentation	Location	Metastasis	Follow-up	Radiated for	Radiation dose	Years after radiation
Chen et al. (37)	F/66	Abdominal pain, nausea, and vomiting	Terminal ileum	Liver	DOD at 14 months	Endometrioid adenocarcinoma / ovary	60 Gy	8
Nanus et al. (36)	F/42	Perforated distal ileum	Distal ileum	Labia majum, vagina, pelvis, urinary bladder, rectovaginal septum, paraortic LNs, lungs, abdomen	DOD at 36 months	Dysgerminoma/ ovary	48 Gy	16
Wolov et al. (39)	F/80	Peripheral edema, abdominal distension, altered bowel function, mucus per rectum	Small and large intestines	Peritoneum, liver	DOD at 2 weeks	Squamous cell carcinoma/uterine cervix	55Gy	20
Wolov et al. (39)	F/69	Anorexia, weight loss, abdominal distension, hematochezia	Small and large intestines	Pleura and peritoneum	DOD at 23th hospital day	Stage IB, grade 3 adenocarcinoma/ uterus	50Gy	7
Berry et al. (10)	M/51	Peritonitis	Small bowel	Pleura	DOD at 5 months	Stage IIA Hodgkin's lymphoma	Total nodal irradiation	3
Su et al. (40)	F/48	NA	Terminal ileum	Liver and local recurrence	DOD at 23thday	Squamous cell carcinoma/uterine cervix	NA	3,2
Hwang et al. (38)	F/60	Anorexia, abdominal pain, abdominal distension	Small intestine	NA	DOD at 2 months	Stage IIIB carcinoma/uterine cervix	96,50 Gy	8
Hansen et al. (19)	F/76	Watery diarrhea, vomiting, abdominal pain, weight loss	Small bowel	Serosal surface of stomach, small and large bowel, liver, spleen, urinary bladder	DOD at 5 months	Endometrial adenocarcinoma/ uterine corpus	45,1 Gy	7
Suzuki et al. (31)	F/61	Intestinal perforation	Terminal ileum	Peritoneum, stomach, liver, spleen, urinary bladder, direct extension to right diaphragm and lower lobe of lung	DOD at 12 months	Squamous cell carcinoma/ uterine cervix	NA	20
Aitola et al. (7)	F/50	Intestinal obstruction	Terminal ileum	Intra-abdominal spread and retroperitoneal recurrence	AWED at 21 months	Stage I endometrial adenocarcinoma/ uterus	55,6 Gy	14
Aitola et al. (7)	F/78	Bowel obstruction	Jejunum	Abdominal wall and retroperitoneum	DOD at 25 months	Endometrial adenocarcinoma/ uterus	55,5 Gy	10
Policarpio-Nicolas et al. (1)	F/51	Decreased appetite, abdominal pain, increasing abdominal girth	Terminal ileum	Peritoneum, liver, appendix	DOD at 10 months	Stage IIB adenocarcinoma/ uterine cervix	50 Gy	9
Karpeh et al. (20)	NA	NA	Terminal ileum	Recurrent retroperitoneal LNs metastases, widespread pelvic disease, vagina and vulva	NA	Dysgerminoma/ ovary	NA	14
Selk et al. (29)	M/57	Abdominal distension, shortness of breath	Small bowel	Peritoneum and chylous ascites	DOD at 4 months	Chondrosarcoma/ right hemipelvis	NA	8

Table I. *(continued)*

Khalil et al. (2)	M/68	Gastrointestinal bleeding, melena, abdominal pain	Small bowel	Peritoneum, celiac LNs, abdominal wall	DOD at 3 months	30 years history of heavy occupational exposure to radiation and polyvinyl chloride	NA	NA
Navarro-Chagoya et al. (42)	M/45	Gastrointestinal bleeding, melena, weight loss, epigastric pain, fever	Small bowel	Omentum	NA	Unknown pelvic tumor	NA	10
Current case	F/72	Abdominal pain, intestinal obstruction	Terminal ileum	None	ANED at 26 months	Uterine leiomyosarcoma	NK	24

M, male; F, female; NA, not available; NK, not known; AWED, alive with evidence of disease; DOD, dead of disease; ANED, alive with no evidence of disease.

epithelioid hemangioendothelioma, Kaposi's sarcoma (KS), epithelioid gastrointestinal stromal tumor, other sarcomas with epithelioid morphology, melanoma, or a poorly differentiated adenocarcinoma. There is an overlap between epithelioid hemangioendothelioma and epithelioid angiosarcoma. In its pure form, epithelioid hemangioendothelioma is usually composed of cords, strands and solid sheets of vacuolated polygonal to round cells embedded in a myxohyaline matrix, but it lacks of a solid growth pattern generally regarded as a diagnostic clue in favor of epithelioid angiosarcoma [15,25]. The histologic features that help distinguish angiosarcoma from KS are the presence of cavernous vessels and epithelioid endothelial cells in the former and the evidence of both intra-extracellular eosinophilic hyaline bodies and immunoreactivity for HHV-8 in the latter. A vasoformative pattern with evident cytologic atypia and immunoreactivity to CD31 and other endothelial markers would strongly argue against epithelioid GIST. The distinction of PRA from poorly differentiated carcinoma, melanoma, epithelioid leiomyosarcoma and proximal-type epithelioid sarcoma is based on immunohistochemistry. There is overlap in immunohistochemical expression between some epithelioid vascular malignant neoplasms and poorly differentiated epithelial tumors with cytokeratin pools demonstating a membranous and citoplasmic reactivity in both tumor types. It has been suggested that CK19 and CK20 could be useful markers in differentiating epithelioid angiosarcomas from carcinomas, which would be CK19 and CK20 positive [9,11]. Finally, epithelioid angiosarcoma may mimic the angiomatoid variant of epithelioid sarcoma, both in morphology and by the occasional expression of cytokeratin. However, angiosarcoma is more pleomorphic and usually expresses CD31 and Factor VIII.

The standard of care for PRA of the small bowel is complete surgical excision, which is often not possible due to infiltrative and multifocal growth pattern of the neoplasm. Given the rarity of the angiosarcoma of small intestines, there are no large trials that provide guidance for systemic chemotherapy. All adjuvant therapy protocols are empiric and based on studies of soft tissue angiosarcoma that suggest paclitaxel, doxorubicin, docetaxel, and thalidomide therapy may have benefit [3,16,17,51].

In conclusion, primary small bowel angiosarcomas are rarely seen and comprise less than 1% of all intestinal neoplasms. Radiation associated small intestine angiosarcoma might be a special type but with a similar outcome. The pathogenesis of these neoplasms is presumed to be due to irreversible DNA damage. The long latency period associated with these tumors is due to a multistep process involving several dominant gene mutations and deletions that accumulate in the genome over a period of time leading to carcinogenesis. Though the exact molecular mechanisms of carcinogenesis of radiation induced sarcomas are unknown, widely accepted theories include the expression of protooncogene c-jun and inactivation of tumor suppressor genes P53 and Rb. Close long-term surveillance is highly recommended in patients undergoing radiotherapy for the development of secondary malignancies, such as angiosarcomas, in the area of radiation in order to diagnose and treat at early stages.

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Case Report

Small hepatic veins Budd-Chiari syndrome and paroxysmal nocturnal hemoglobinuria - The association of two rare entities: a case report

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Summary

Small hepatic veins Budd-Chiari syndrome is a rare disorder characterized by hepatic venous outflow obstruction limited to the small intrahepatic veins, with normal appearance of the large hepatic veins at imaging. In this case only a liver biopsy can demonstrate the presence of a small vessels outflow block. Paroxysmal nocturnal haemoglobinuria (PNH) is one of the most severe acquired thrombophilic state and represents one of the main aetiological factors of Budd-Chiari syndrome. In patient affected by PNH with liver impairment and/or ascites, Budd-Chiari syndrome must be always taken into consideration and, if necessary, a liver biopsy performed to exclude the small hepatic veins involvement. We report a case of small hepatic veins Budd–Chiari syndrome secondary to paroxysmal nocturnal haemoglobinuria.

Abbreviations:

BCS: Budd-Chiari syndrome, PNH: paroxysmal nocturnal haemoglobinuria, PIG: phosphatidylinositol glycan, INR: international normalized ratio, MRCP: magnetic resonance cholangiopancreatography, NASH: non-alcoholic steatohepatitis, NAFLD: non-alcoholic fatty liver disease, CT: computed tomography.

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Introduction

Budd-Chiari syndrome (BCS) is a rare disease characterized by hepatic venous outflow obstruction at each level from the small hepatic veins to the atrio-caval junction. In small hepatic veins Budd-Chiari syndrome the obstruction is limited to the small intrahepatic veins, with normal appearance of large hepatic veins. In this case, liver biopsy is necessary for diagnosis. The major risk factors of BCS are thrombophilic conditions [1]. Paroxysmal nocturnal haemoglobinuria (PNH) is an acquired clonal blood disorder affecting hematopoietic stem cells that is caused by a somatic mutation in the phosphatidylinositol glycan (PIG)-A gene. Principal manifestations are intravascular hemolysis and thromboembolism. Current treatment for PNH includes eculizumab, a monoclonal antibody direct to complement factor C5 blocking intravascular haemolysis and reducing thrombotic events in PNH, and it is also the best prophylaxis [2,3]. We describe the case of a 50-years-old man with the diagnosis of paroxysmal nocturnal haemoglobinuria from 2014 in therapy with eculizumab, administered at the dosage of 900 mg once every two weeks. In October

2017, he was submitted to the replacement of biological aortic valve and ascending aorta prosthesis for the treatment of aortic steno-insufficiency. After the surgical procedure, blood tests documented hyperbilirubinemia: total bilirubin 47 mg/dl (normal 0.3-1.2 mg/dL) and direct bilirubin 29 mg/dl. In December 2017 he was hospitalized for the occurrence of jaundice. Laboratory analyses revealed elevation of total bilirubin of 29 mg/dL and direct bilirubin of 22 mg/dl, of gamma-glutamyl transferase (185 U/L; normal 5-36) and alkaline phosphatase (254 U/L: normal 35-104), INR was 2.2. An abdominal ultrasonography and a magnetic resonance cholangiopancreatography (MRCP) showed cholecystolithiasis and choledocholithiasis, portal vein diameter of 18 mm and spleen diameter of 18 cm. Common bile duct stones were removed using endoscopic sphincterotomy. Despite the treatment of biliary obstruction, blood levels of bilirubin decreased but persisted over upper normal values. All causes of liver disease (alcohol, drugs, viral, autoimmune, hemochromatosis, Wilson disease, NASH/NAFLD) were ruled out. Moreover, also the hypothesis of a liver disease secondary to congestive heart failure was ruled out by echocardiography. To better define the etiology of the hyperbilirubinemia the patient was transferred to our Unit (Hepatology) where a new abdominal ultrasonography was performed showing hepatomegaly, regular liver surface, inhomogeneous liver parenchyma, ascites and patency of the portal vein. The hepatic veins were thinned but patent and the flow was detectable at Doppler ultrasound; liver stiffness measurement showed different values in the different hepatic segments, ranging from 13 to 17 KPa. Blood test documented also low values of albumin (2.4 g/dl), pseudo-cholinesterase 2200 U/L and prolonged prothrombin time (INR 2.3). A contrast enhanced CT-scan confirmed the patency of the hepatic veins and, therefore, a percutaneous liver biopsy was performed. On histology the liver parenchyma showed sinusoidal dilation with marked perisinusoidal fibrosis (Fig. 1A). There was evidence of organizing occlusive thrombosis of centrolobular veins (Fig. 1B). Focal hemorrhages were observed, associated with small foci of hepatocellular necrosis.

To exclude a further cause of thrombophilia in addition to PNH already in pharmacological treatment, an extensive screening for hereditary and acquired thrombophilia was performed and it was negative. Thus, low molecular weight heparin was added to eculizumab therapy but immediately stopped because of the development of melena. An upper endoscopy excluded the presence of gastroesophageal varices and therefore the gastrointestinal bleeding was attributed to the previous sphincterotomy. During hospitalization, the patient developed fever and blood culture isolated methicillin and daptomycin-resistant *Staphylococcus aureus*. A diagnosis of endocarditis with multiple spleen infarctions and periprosthetic aortic abscess was reached and targeted antibiotic therapy (i.v. vancomycin and ceftarolin) was started. Consequently, ascites became less responsive to diuretic therapy, renal function worsened, and the patient was treated with repeated paracentesis and intravenous human albumin. Despite the adequate antibiotic therapy, the patient died for the diffusion of the infection.

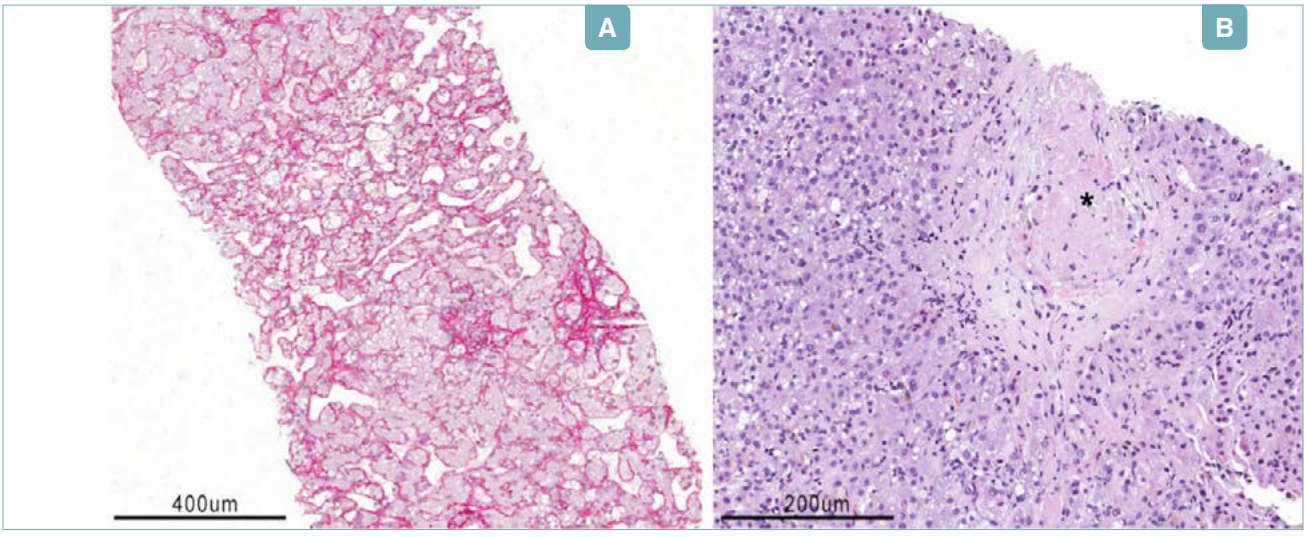

Figure 1. (A) Liver biopsy shows sinusoidal dilation with marked perisinusoidal fibrosis (picrosirius red staining); (B) Organizing occlusive thrombosis (asterisk) of centrolobular vein (hematoxylin and eosin staining).

Discussion

Paroxysmal nocturnal haemoglobinuria is a rare and life-threatening acquired haematological disorder. Budd-Chiari syndrome is a rare vascular liver disease, and, of it, the small vessels Budd-Chiari syndrome represents an even more rare form. However, PNH is considered the most severe acquired thrombophilic state and represents one of the main aetiological factors of vascular liver diseases. In particular it is responsible of the 19% of cases of Budd-Chiari syndrome [4] justifying why the diagnostic work-up suggested by the current guidelines includes the active search for PNH in patients with BCS.

On the other side, in patients affected by PNH the presence of signs of liver disease and in particular the occurrence of jaundice, ascites and alteration of liver function tests should raise suspicion of BCS. Nonetheless, PNH is characterized by chronic intravascular haemolysis that predisposes to the development of bilirubin gallstones often complicated by biliary obstruction [5] which could further confuse the clinical picture. In fact, in our patient, the hyperbilirubinemia could have been correctly attributed only to the presence of cholecystolithiasis and choledocholithiasis and to the increased hemolytic attacks consequently to the surgical procedure. However, the persistence of so high levels of bilirubin despite the endoscopic treatment and the presence of ultrasonographic features suggestive for Budd-Chiari syndrome (ascites, hepatomegaly, inhomogeneous aspect of liver parenchyma) lead us perform liver biopsy. Again, as in our case, in presence of a high suspicion, the patency of hepatic veins should not lead to exclude the diagnosis of BCS but to search for small hepatic veins BCS. Eculizumab is a humanized monoclonal antibody that targets the terminal complement protein C5 and inhibits terminal complement-mediated haemolysis associated with PNH and it reduces the risk of clinical thromboembolism these patients [6]. These facts strongly suggest that the main cause of thrombosis in PNH is complement activation and/or haemolysis [7]. Therefore, current guidelines do not suggest any anticoagulant treatment for prophylaxis of deep vein thrombosis in PNH patients treated with eculizumab. Once a venous thrombotic event occurred, anticoagulant therapy is necessary to solve the thrombosis and to prevent new events [8]. The demonstration of a concomitant BCS and so of the thrombosis of hepatic or small intrahepatic veins requires the start of anticoagulants once the complications of hypertension have been treated. Maybe, in our case, Budd-Chiari syndrome was already present before the start of therapy with eculizumab.

Finally, the more relevant clinical problems of our patient and, after, the cause of his death were the endocarditis and the periprotesic aortic abscess that were not related to the liver disease. Inhibiting the complement protein C5, eculizumab make the patients more susceptible to infections. Thus, the prophylaxis, the prompt diagnosis and the timely treatment of the infection is a crucial point of the management of the patients affected by PNH and in therapy with eculizumab [9].

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Case Report

Atypical fibroxanthoma/pleomorphic dermal sarcoma of the scalp with aberrant expression of HMB-45: a pitfall in dermatopathology

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Summary
Atypical fibroxanthoma (AFX) has been considered as the non-infiltrating precursor lesion of pleomorphic dermal sarcoma (PDS), which shows an aggressive clinical behavior, because of its extensive invasion of the deeper skin layers. Although these two tumors may represent two stages of the same disease, it can be difficult to differentiate between them, because of their similar clinical and histological features [1]. Furthermore, they must be distinguished from a spindled variant of squamous carcinoma, melanoma and leiomyosarcoma [2]. AFX/PDS still remains a diagnosis of exclusion, that needs to combine immunohistochemical markers for a definitive diagnosis. Usually AFX/PDS shows positivity for CD10, CD99, CD68, vimentin and lysozyme, while S100, HMB45, MART-1, cytokeratins, CD34, CD31, desmin and h-caldesmon are absent.
We report a case of 89-year-old male, with a history of squamous cell carcinoma removed from the right ear, presented to our department with a recently growing, ulcerated and bleeding 2 cm nodule on the scalp. After surgery the tumor recurred with infiltration to the cranial theca. The final histological diagnosis was "pleomorphic dermal sarcoma" (PDS), which showed an unexpected positivity for HMB45. We present, to the best of our knowledge, the first case of AFX/PDS with an aberrant diffuse expression of HMB45 and an aggressive biological behavior, that leads us to a difficult exclusion diagnosis.

Key words: atypical fibroxanthoma, HMB45, pleomorphic dermal sarcoma

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Introduction

Since 1960, when Helwing described for the first time AFX as a cutaneous tumor with marked pleomorphism, but with a benign course, the entire spectrum of AFX and related neoplasms have been a topic of ongoing debate [3-7].
In 1964, Kempson defined AFX as histologically malignant, but biologically benign. In 1991, Murphy and Elder consider AFX as a fibrohistiocytic neoplasm of the skin, showing malignant histological features and locally aggressive course, fitting with a low-grade variant of "malignant fibrous histiocytoma" (MFH) [8,9].
Improperly and for many years the terms AFX and MFH were used interchangeably or referring to superficial (AFX) or deeper (MFH) tumors. The term MFH has been supplanted by the term "Undifferentiated pleomorphic sarcoma" (UPS). Tumors that in the past have been referred to as cutaneous UPS, or as superficial MFH, are now defined as Pleomorphic Dermal Sarcoma (PDS) [7,10,11].

AFX and PDS are rare mesenchymal tissue tumors that are clinically and morphologically similar, which can be considered two stages of the same disease, rather than two different entities. AFX is considered the non-infiltrating precursor lesion of PDS which shows an aggressive behavior and high grade of malignancy [2,12]. Often the diagnosis of AFX and PDS remains a diagnosis of exclusion, because of the absence of discriminatory morphological or immunohistochemical features [7].

Case report

In January 2017 a 89-year-old male, with a history of squamous cell carcinoma removed from the right ear, presented to our department with a recently growing nodular, ulcerated and bleeding nodule on scalp, measuring 1 cm x 2 cm. Excision was performed and histopathological examination showed a dermal highly vascularized, ulcerated tumor composed of atypical spindle cells, arranged in fascicles, elongated nucleus, often in mitosis (Fig. 1). Immunohistochemistry showed tumor cells negativity for S100 protein (Fig. 2), cytokeratins (AE1-AE3- 34 betaE12), smooth muscle actin, desmin, p16, CD31, CD34, Melan-A, SOX10, tyrosinase. Vimentin, CD10 (Fig. 3), CD68, p53, HMB45 were positive (Fig. 4) and Ki-67 expression rate was 50%. Sometimes Perls positive cytoplasmic granules (hemosiderin deposits) and Fontana-Masson positive blackish granules (melanin deposits) were detectable.
Based on immunomarking squamous cell carcinoma (negativity of cytokeratins, 34betaE12 and p16), leiomyosarcoma (negativity of smooth muscle actin and desmin), superficial dermatofibrosarcoma protuberans (negativity of CD34) and melanoma (negativity for S100 protein, Melan-A and tyrosinase) was excluded, although there was an unexpected cytoplasmic positivity for HMB45. In the presence of CD10 and CD68 positivity a diagnosis of atypical fibroxanthoma with aberrant expression of HMB45 was done. Wide excision was performed, but due to the size of the tumor

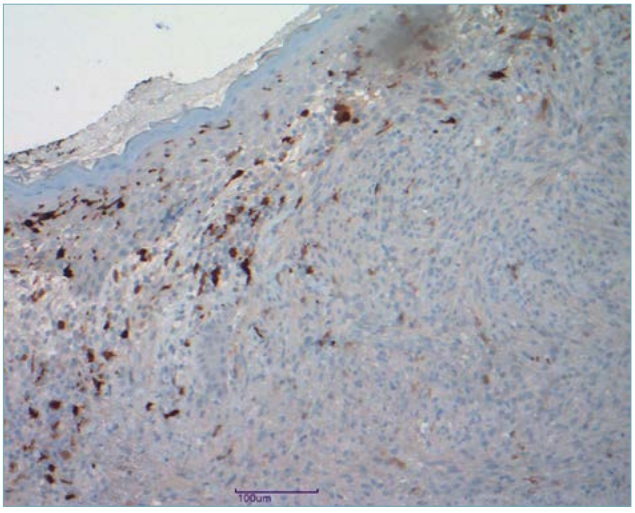

Figure 2. Immunohistochemical analysis showing tumor cells negativity for S100 protein (10x).

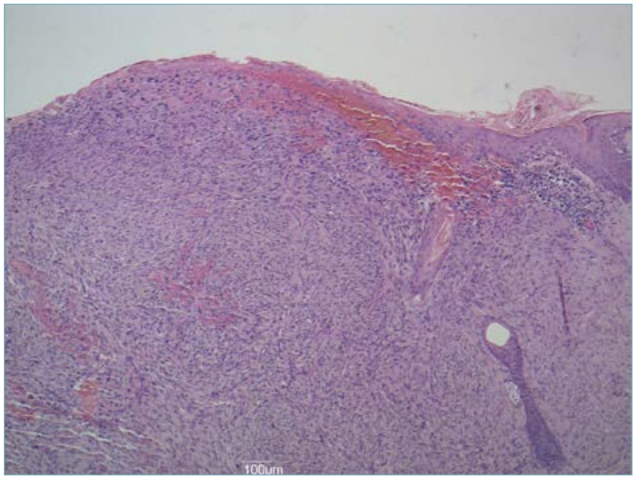

Figure 1. Dermal highly vascularized, ulcerated tumor composed of atypical spindle cells, arranged in fascicles, elongated nucleus, often in mitosis (H&E staining: 10x).

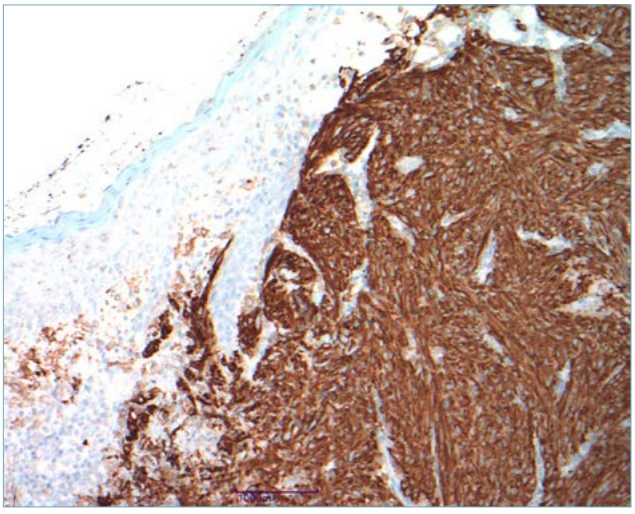

Figure 3. Diffuse positive staining with CD10 (10x).

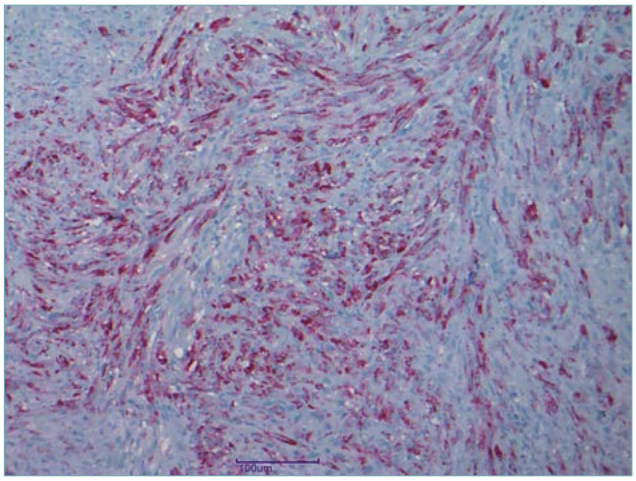

Figure 4. Aberrant cytoplasmatic positivity with HMB45 staining (20x).

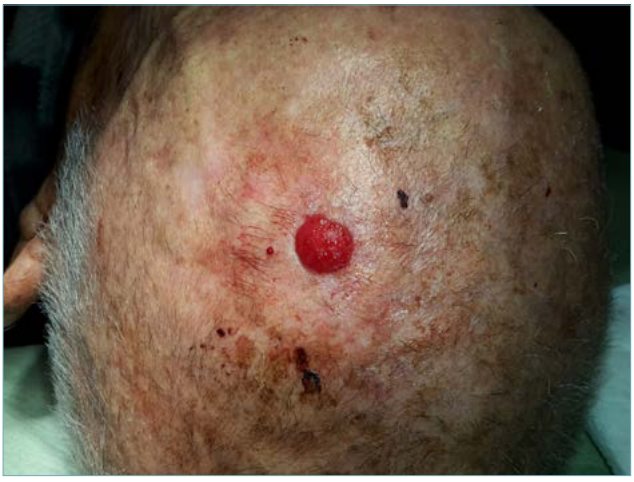

Figure 5. Clinical recurrence on the patients' scalp after wide surgical excision.

that reached the lateral margins of surgical resection, the tumor recurred with invasion of the cranial theca (Figs. 5, 6). Chest radiography and neck lymph nodes ultrasound were negative.

We referred the patient to the department of plastic surgery in order to perform radical surgery and final histological diagnosis, based on infiltration of the cranial theca, was PDS with aberrant HMB45 expression. Molecular biology investigation on BRAF V600E was performed to complete the histological study and was negative.

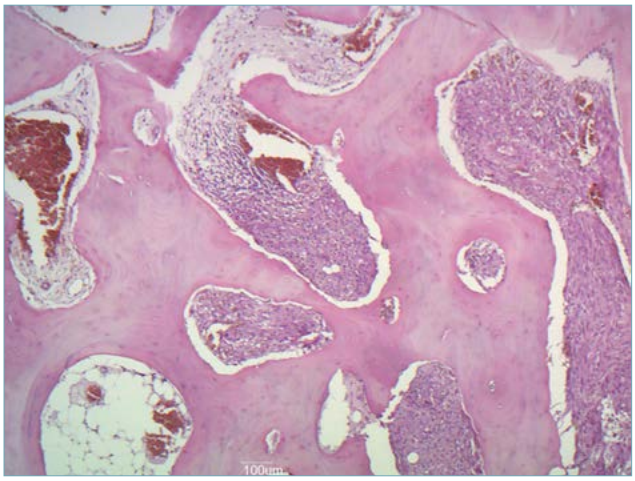

Figure 6. Recurrent tumor with invasion of the skull (10x).

Discussion

AFX is an uncommon cutaneous neoplasm, with uncertain etiology, that mainly arises from either fibrocytic or myofibrocytic cells [13]. It represents up to 0.2% of all skin tumors and has a male predominance (70%). Commonly the tumor occurs on sun damaged skin of elderly people, between 7th and 8th decade of life [5, 6, 14-16]. Usually it is described as a solitary, exophytic, painless, firm, skin-colored or brown-red, often eroded or ulcerated nodule. It can grow up rapidly, but the tumor size rarely exceeds 2 cm. Head and neck are involved in the 80% of cases (especially the scalp). The lesions might also appear on ears, cheeks and nose, while on forearms or on dorsa of hands are rare. Histologically, tumor cells are located in the dermis with sometimes a grenz zone present between epidermis and tumor, but more commonly tumor reaches the epidermis, that can be atrophic and ulcerated. Tumor cells show spindled and epithelioid features with pleomorphic vesicular or hyperchromatic nuclei. Usually giant cells are present. Mitoses are often numerous and may show atypia. The most common morphological pattern is dominated by spindle cells mixed with epithelioid cells, followed by variants with predominantly spindle cells, exclusively spindle cells, or predominantly epithelioid cells. Unusual variants of AFX exist. They include clear cell, osteoid, osteoclastic, chondroid pigmented, granular cell variant. There are not necrosis or vascular invasion [3,5,15-18]. Atypical fibroxanthoma has been considered as a precursor lesion of PDS which shows an aggressive clinical behavior. Although those two tumors may represent two stages of the same disease, it can be difficult to differentiate between them,

because of their similar clinical and histological features. The only morphological features that allow us to distinguish that two lesions are the larger tumor size, deeper subdermal involvement, invasion of fascia or muscle, lymphovascular or perineural invasion and/or necrosis [2,10]. Furthermore, the bizarreness and atypia seen in AFX and PDS cells might suggest a diagnosis of malignant tumor, for that reason it is vital to distinguish AFX/PDS from other cutaneous neoplasms for diagnosis and management. The main histological differential diagnosis is a spindled variant of squamous carcinoma, melanoma (especially spindle or desmoplastic type), or leiomyosarcoma. Immunohistochemistry can help in this distinction [3,14-18].

Considering that in AFX/PDS several morphological features overlap those found in mesenchymal epithelial malignant tumors, immunohistochemistry assumes a very important role in the differential diagnosis of AFX/PDS. AFX shows a positive reactivity to vimentin, CD10, CD68 and sometimes actin, whereas it is usually negative for CAM5.2, CD34, Melan-A, S100, HMB45, cytokeratins A1/A3 14. Indeed, focal staining for single additional melanocytic marker has been reported [19,20].

Based on these considerations, AFX/PDS still remains a diagnosis of exclusion, which needs to combine immunohistochemical markers for a definitive diagnosis. Usually AFX/PDS show positivity for CD10 (in 95-100% cases), CD99, CD68 (in more than half cases), vimentin and lysozyme. Sometimes smooth muscle actin expression has been reported in some AFX. While S100, HMB45, MART-1, cytokeratins, CD34, CD31, desmin and h-caldesmon are absent in AFX/PDS. CD10 (or the common acute lymphoblastic leukaemia antigen, CALLA) is considered a useful marker for AFX (positive in 95-100% of cases), but also 1/3 of malignant melanomas, about half cases of squamous cell carcinomas and half cases of leiomyosarcomas are positive [3,6,21,22]. CD99 is a glycoprotein, indicative of myofibroblastic differentiation with a positive rate in AFX between 35% and 73% [19,20]. However, malignant melanoma (10-60% cases) might show a positivity for CD99, but no cases of SCC positive for CD99 have been reported. CD68 is positive in more than half of all AFX, but it is also detectable in 86% of malignant melanoma. Procollagen-1 was strongly positive in 87% of AFX cases, but also in 1/3 of the desmoplastic malignant melanomas and desmoplastic squamous cell carcinomas. AFX is distinguished from leiomyosarcoma, which has a smooth muscle differentiation, with positivity for calponin and h-caldesmon [5]. Cytokeratins are epithelial markers, positive in squamous cell carcinoma, very useful to exclude sarcomatoid or spindle cell SCC. S100 protein, used to mark Schwann cells, is a melanogenesis marker, used for initial screening for melanocytic tumors, positive in melanoma. HMB-45 and MART-1 are melanocyte specific markers to confirm the melanocytic nature of S100 positive lesions [10]. However, poorly differentiated SCC might not express cytokeratins and not all melanomas show staining positivity for S100 and HMB45. Furthermore, sometimes also AFX may express focal positivity for S100, because of dendritic Langerhans cells entrapped inside [6,23]. Moreover, in the literature two cases of AFX, which show focal expression of HMB45 and MART1, are described [17,24]. Therefore, caution is advisable in interpreting focal and slight positivity for additional melanocytic markers [19,20]. We presented a similar case of AFX with aberrant expression of HMB-45, and we reported theories suggested by Smith-Zagone et al. concerning for reason of HMB45 expression in a non-melanocytic neoplasia. HMB-45 recognizes the gp100, whereas MART-1 recognizes a protein called PMe117. Both proteins are localized in the inner membranes of premelanosomes. They are not specific only for the melanocytic lineage, they are positive in angiomyolipomas, lymphangioleiomyomatosis, tumors of the lung, adrenal cortical tumors and sex cord tumors of gonads. This may be explained by the presence of premelanosomes in some of these tumors or by an antibody cross reactivity against an antigenic epitope similar to gp100 in steroidogenic tumors. In AFX premelanosomes are not demonstrated, consequently the positivity could be explained by a cross reactivity with an antigen similar to that observed in steroidogenic tumors, which do not contain premelanosomes. The expression seems to be more important in the cells with vacuolated cytoplasm, suggesting that a lipid could be possibly considered the cross- reacting substance. Another theory could be that some of these tumor cells have phagocytized fragments of melanocytes [17,24].

Conclusion

We present, to the best of our knowledge, the first case of AFX/PDS with an aberrant diffuse expression of HMB45 and an aggressive biological behavior, which led to a difficult diagnosis of exclusion [14]. It is critical to distinguish AFX from PDS, because of their clinical and morphological similarities. Often it is difficult to distinguish those two entities, considered to be the two extremities of the same clinical-pathological tumor spectrum, especially without evidence of extensive invasion of the deeper layer [7]. The clinical progression of our case supports that AFX could be actually considered as the initial lesion of a spectrum

of a tumor, in which PDS represents the final manifestation. For that reason we agreed to call it "cutaneous pleomorphic sarcoma" or with a more appropriate name "AFX/PDS" [11,12].

According to our experience, we regard S100 as the most important stains in the main differential diagnosis of AFX and melanoma. We suggest considering HMB45 as a non-specific marker to exclude the diagnosis of AFX, in order to avoid diagnostic mistakes. If there is a correlation between HMB45 positivity and AFX evolving to the PDS is yet to be assessed on a larger series.

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Pathologica Storica

Spanish flu in Turin as told by historical autopsy reports

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Summary

Spanish flu spread worldwide between 1918 and 1920 causing over 20 million deaths, exceeding even the number of deaths registered during the First World War (WWI). The main symptom of the disease was hemorrhagic tracheobronchitis, the onset of which was typically sudden and fatal. Young, healthy people died quickly. Despite the tragic impact of the disease on populations, already exhausted by the First World War, there is very little documentation. This was likely due to the severe censorship of the time. For this reason, autopsy reports can be a relevant source of information on the disease. Historical catalogues kept in Turin, where all autopsies were detailed, can be consulted. According to the "Regolamento di Polizia Mortuaria" dating back to 1892, autopsies were to be performed on all patients that died at home or in hospital. Therefore, autopsy reports showing the spread of diseases among the population can also help us obtain information about the spread of Spanish flu in Turin. While not documented, almost certainly the "Regolamento" was improperly implemented since just 45 cases of Spanish flu were reported, while deaths were most certainly daily and in their hundreds. According to autopsy reports, the first case occurred on 8th October 1918, although, the first official diagnosis is dated as being 24th November 1918. The records show that 18 people died during the first Italian pandemic wave. The second Italian pandemic wave seems to have been even more aggressive in Turin with 27 people having died between 8th January 1920 and 7th February 1920.

Key words: history of pandemic, Spanish flu, ancient autopsy reports

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Introduction

The Spanish flu pandemic spread worldwide between 1918 and 1920 and exceeded the number of deaths registered during the First World War [1]. It was probably, along with the Plague and the Black Death, one of the worst pandemics in history [2]. More than one-third of the global population of 500 million was affected and around 50-100 million people died [3]. There are many different theories about the spread of this pandemic. The case of a military cook based in Camp Funston, Kansas in March 1918 is generally referred to as being the first reported occurrence of Spanish flu [4], according to other reports the real centre of the pandemic began in a major troop staging and hospital camp in Etaples in France in late 1917 [5], while, other reports suggest that the epicentre of the flu could be traced to in China [4]. Wherever the illness originated from, in the US, France or China, it certainly did not originate in Spain, as the name would suggest. The pandemic spread quickly worldwide between the spring and summer of 1918. The first pandemic wave especially affected military troops. Notably, the mortality rate of the first wave was lower than the second. While poor health and sanitary conditions of the populations by

the end of World War I were surely an influencing factor of the disease, King Alfonso XIII of Spain became ill in neutral Spain, suggesting that these factors were not decisive. In late spring of 1918, the Spanish press agency Fabra Reported that "A strange form of the disease of epidemic character, has appeared in Madrid. The epidemic is of a mild nature, with no deaths reported". This was the first official report of Spanish Flu in Europe. Spain was a neutral country during the war and therefore the press there was not censored [6,7]. This first report, however, gave the impression that the flu had started in Spain and thus it was familiarly called Spanish flu. Diversely in Spain, it was called "The French flu" or "The Naples Soldier" from the title of a popular musical. The press in countries directly involved in the fighting were not permitted to report on the spread of the disease and due to the censorship, initially, measures against the spread of the disease were not taken. At the end of the summer of 1918, with the epidemic becoming more aggressive, people began to die. Italy had the hightest number of cases of contamination after Russia, but in terms of death rate, Portugal's toll was even higher [8]. According to Giorgio Mortara, the deaths in Italy were about 600,000 during the three waves of flu [9]. The first wave began at the end of April 1918, lasted until June and then disappeared in July 1918. The disease in this first wave was mild and mortality was low. In September 1918, the second wave began and the flu spread mostly in the centre and south of Italy up until May 1919. Spanish flu had its own W-shape of mortality. It typically affected young healthy people between 20 and 40 years of age and not only old people and children [10]. This was probably due to immunity acquired by older people in previous similar flu pandemics. It may also have been due to the major reactivity of young people's immune system, according to the theory of "cytokine storm" which was perhaps the peculiar pathogenic mechanism of the Spanish flu [11]. The main symptom of the disease was hemorrhagic tracheobronchitis, which would appear suddenly and was usually fatal. The cause of the flu was most likely a virus similar to that of H1N1 influenza strains [12]. The genomic sequence of the virus was finally studied in 1997 using fixed and frozen tissues from influenza victims [13]. At the time of the spread of the pandemic, the Director of paediatrics at the University of Genoa, Pacchioni wrote in his "Pathologica" in 1919 that the pathogen responsible for the pandemic could be a very virulent variant of Pfeiffer's bacillus in association with Streptococci [14]. On the other hand, Doctor Segale wrote in the same year about the discovery of a new pathogen: Streptococcus pandemics [15] and its role in the pandemic [16]. In Turin, Professor Pio Foà believed that the pandemic was due to Streptococcus pandemic and not to Pfeiffer's bacillus [17]. Despite the freedom of scientific debates, the censorship forbade the spreading of news about the pandemic and this certainly helped to spread the disease both in Italy [18] and abroad [19]. The debate in the scientific milieu was very intense, although censorship and self-censorship prevented a public discussion of the causes of the pandemic. The censorship was due to wartime ideology; the disclosure of bad news was forbidden in order to avoid demoralising the army. The self-censorship among doctors was instead due to the difficult identification of the aetiological agent of the Spanish flu. Indeed, this difficulty seemed to end the golden age of microbiological discoveries causing a kind of embarrassing self-censorship among the medical community [20]. According to records, the pandemic ended in the Western Hemisphere in May 1919. In Japan, there was a third epidemic wave between the end of 1919 and 1920 [21].

Materials

The general scarcity of information due to censorship increases the importance of the little information that is available [22]. Archive research was carried out to find cases of Spanish flu among the autopsy reports in the Royal Institute of Pathology of Turin and which are now kept by the Institute of Pathology of Turin. The aim of the research was to reconstruct the history of the Spanish flu in Turin according to these reports. The reports generally describe the main pathological findings and the main cause of death. Together with this data, there are some other details such as the name of the patient, when they died, when the autopsy was performed and the hospital they died in. Among all the autopsy reports falling between 1918 and 1920, those with the final diagnosis of "flu" were selected. The cases having pathological findings pointing to pandemic flu were also selected. The main pathological findings selected for these studies according to reports of autopsies performed in Italy were haemorrhagic trachea bronchitis and haemorrhagic pneumonia [23].

Results

The retrospective study on autopsy reports allowed for the discovery of the first autopsy performed on a victim of Spanish flu in Turin. This was a 35-year-old woman who had died on 8th October 1918. According to the main historical documentation, no cases occurred during the first pandemic wave of Spring 1918, and therefore it is considered that this first case be-

longs to the second pandemic wave. The descriptions of the cases of Spanish flu show typical symptoms suggesting pandemic flu along with the presence of haemorrhagic tracheobronchitis described as being "very violent". According to the descriptions, the clinical presentation and the pulmonary involvement typical of Spanish flu was so serious that some patients died within a few hours after the onset of symptoms. Similar qualitative or clinical descriptions were highly unusual in common autopsies and suggest particular attention of the pathologists working on these cases and are likely due to the dramatic clinical history of these patients. Moreover, because of the strict censorship of the time, pathologists avoided including a written diagnosis of pandemic flu in their conclusions, even if the diagnosis was clear enough, as in the case of the first victim. Indeed, after the first victim, 8 more cases occurred in the following days, all of them showing the same pathological findings typical of Spanish flu, but none reported the pandemic as being the final cause of death. Only on the 24th of November 1918 do we find the fatal diagnoses written as being "Bronchopneumonia due to flu". This, not surprisingly, was registered after the end of the war. The first victims of the first pandemic wave were all among the young, mirroring the general age of victims around the world. The youngest victim was a 10-year-old child, although a report also mentions the miscarriage of a mother suffering from bronchopneumonia. In the following months, 18 cases were described. The pandemic wave went on until April 1919, and records show a decreasing number of victims. The last case, in spite of the final diagnosis, was probably not Spanish flu. The diagnosis is recorded as being "Bronchopneumonia due to flu" but the pathological findings describe bronchopneumonia without haemorrhage. Although the mutation of the pathogenic agent over the weeks is a possible explanation, it is more probable that this case was common bronchopneumonia with some symptoms of flu. After 12th April 1919, no autopsy report having a diagnosis of Spanish flu were found. The second pandemic wave was over in Turin too. According to autopsy reports the victims were 18. More realistically there were possibly hundreds of deaths each day. It is generally accepted that the pandemic flu was over in the northern hemisphere after the second pandemic wave. It is also accepted that this pandemic wave was overall the worst, some authors even suggesting that this was the real reason the First World War came to an end. Surprisingly, the worst pandemic wave in Turin occurred in the winter of 1920 (Fig. 1). On 8th January 1920, an autopsy on a 62-year-old man was performed. A short clinical history is also given; he arrived at the hospital in agony and died just two hours later. It was also reported that he showed clinical symptoms of heart failure. However, the presence of pneumonia and particularly the red colour of the mucosa of the bronchi allows us to reasonably conclude that this case is among those of Spanish flu. The pathological findings are surely those of pandemic flu, but no diagnosis of Spanish flu was made. Probably the pathologists believed that the pandemic was over and therefore did not recognize this case as being due to the pandemic. The presence of this short clinical history is, however, very significant because it shows that some pathologists likely found these sudden deaths more impressive than the others. The same diagnosis is given in the following three autopsy reports, all dating back to 8th January 1920. On 14th January 1920, the first diagnosis of Spanish flu during a third pandemic wave was made. The victim was an 18-year-old woman who arrived at S. Giovanni Hospital in agony with severe dyspnoea and who died within a few hours. The pathological findings were haemorrhagic bronchopneumonia, congestion of the liver and the initial stages of reactive splenic hyperplasia. The pandemic was back. The second pandemic wave in Turin was the worst one in terms of the number of victims: 27 people and one foetus died between 8th January 1920 and 7th February 1920. Some very particular cases can be seen, such as that of a 40-year-old man who collapsed in the street and promptly died. He had probably been suffering from the onset of Spanish flu, and pneumonia and congestion of the lungs were evident. The patient's spleen did not show hypersplenism because it was already chronically enlarged. Another curious case is that of an autopsy performed during a lecture on autopsy techniques held by Professor Pio Foà, highlighting that there were no particular concerns about the post mortem infectivity of the flu.

Professor Pio Foà wrote some notes about Spanish flu in his book on pathology. He believed in the theory of the bacteriological pathogenesis of the flu, as reported in the paper of Segale and published in "Pathologica" in 1919. In Foà's words, the pandemic flu seemed, in fact, in consideration of the scientific analysis of the disease, to be less dramatic than generally perceived. All 27 cases showed similar pathological findings: haemorrhagic pneumonia, variable hypersplenism and congestion of the kidneys and liver. No other pathological findings were found. The age group having the highest number of victims was between 20-30 years (12/27), although older age groups were also affected. The oldest victim was a 63-year-old. Considering gender, the victims of the first pandemic wave in Turin were in higher percentage female, whereas during the second pandemic wave this statistic was inverted.

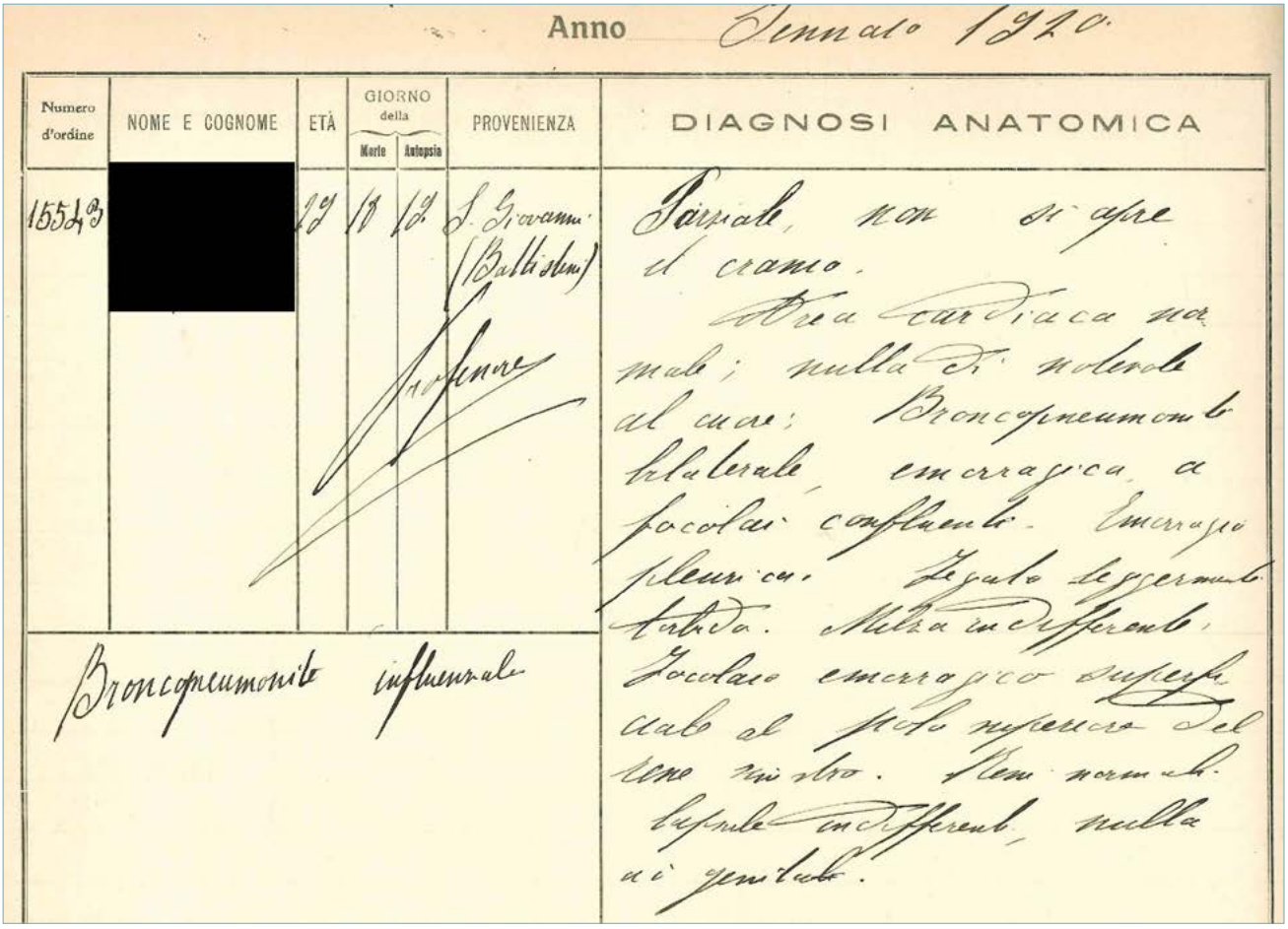

Figure 1. An original autopsy report.

Discussion

The retrospective study of these old autopsy reports shows a distribution of the cases of Spanish flu in Turin and agree with sources of literature regarding gender and age. The number of autopsies carried out during the pandemic period does not reflect in any way the actual number of victims. The reason for this discrepancy may have been caused by wartime censorship. However, it must be also said that no significant increase was reported after the end of the war, despite censorship being lifted. The existence of a number of cases of Spanish flu being diagnosed after the end of the war would seem to exclude an attitude of self-censorship of pathologists. The only autopsy law cited in autopsy catalogues is the "Regolamento Speciale di Polizia Mortuary". According to this law, dating back to 1892, autopsies were to be performed on all people who died at home and all those who died in hospital. The number of autopsies, however, seems to be far too low to allow us to believe that this law was implemented. More realistic is the hypothesis that autopsies were performed only on selected patients, probably on demand by clinicians. Despite the limited number of cases, the most surprising data is the high number of victims during the so-called third pandemic wave since it is generally accepted that there were no more victims after the end of the second European pandemic wave. In Turin, the distribution of cases seems to be quite different. No clear reason can be given for this. The symptoms described on the autopsy reports and the pathological findings are so suggestive of Spanish

flu that they do not allow for the hypothesis of a diagnostic error. The increased attention of pathologists to these last cases of Spanish flu probably depended on the increased clinical attention to Spanish flu and to lethargic encephalitis that coexisted in the same period, therefore, more autopsies than usual were requested by clinicians.

The archive research carried out in the autopsy reports at the University of Turin shows some new and until now unknown aspects of the spread of Spanish Flu. The pathological findings are common, as reported in other sources of literature; gross lesions as well as haemorrhagic tracheobronchitis, however, attention is paid to the congestion of liver and kidney too. This pathological finding is described as "typical of flu", other observations for this are not noted. This suggests that the observation was common, even though there is no further accurate written description of the macroscopic findings. To find detailed descriptions of the microscopical findings we need to refer to the papers of the series of autopsies published in Pathologica [20]. At the end of the pandemic, the presence of catarrhal pneumonia became more common. Perhaps by the end of the pandemic, the pathogenic agent of the flu had mutated, even if its clinical presentation was similar. Therefore the clinical diagnosis was "flu", even if the pathological findings were different. The last autopsy on an accepted case of Spanish flu in Turin was performed on 3rd February, whereas the last case diagnosed as flu dates back to 7th February 1920, although this wasn't Spanish flu since there is no report of haemorrhagic pneumonia. Irrespective of the low number of autopsies reported, and even in the impossibility of knowing what kind of information they actually had, the pathologists of Turin seem to have been experts on pathological findings. These brief clinical histories are very informative in terms of the sudden clinical presentation of the flu and are an important further source of information in consideration of the general scarcity of documentation available. The history of the Spanish flu in Turin has now more clinical, epidemiological and pathological details. This research improves the value of historic autopsy reports in understanding the epidemiology of diseases among the population, even in consideration of the small number of people on which these autopsies were performed.

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