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Melanocytic nevi and non-neoplastic hyperpigmentations

Drug-induced gastrointestinal injury (DIGI).
Updates, reflections and key points

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the next frontier for pathologists
Updated Information for Authors including editorial standards for the preparation of manuscripts

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Text and individual tables must be stored in separate files.
The article must include:
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(3) a set of key words (in English);
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The second page should contain the abstract. At the end of the text should appear the bibliography, the legends to the tables and figures, and specification (where applicable) of the congress where all or part of the data in the paper may have already been presented.

Tables
Must be limited in number (the same data should not be presented twice, in both the text and tables), typewritten one to a page, and numbered consecutively with Roman numbers. In the text and legend of the tables, Authors must use, in the exact order, the following symbols: *, †, ‡, ¶, **, ††, ‡‡ …

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Send pictures in separate files from text and tables.
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The references must be limited to the most essential and recent citations, identified in the text by Arabic numbers and listed at the end of the manuscript in the order in which they are cited. The format of the references in the bibliography section should conform with the examples provided in N Engl J Med 1997;336:309-15. The first six Authors must be indicated, followed by et al. Journals should be cited according to the abbreviations reported on Index Medicus.

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Acknowledgements and information on grants or other forms of financial support must be cited at the end of the references.

Notes to the text, indicated by an asterisk or similar symbol, should be shown at the bottom of the page.

Mathematical terms, formulae, abbreviations, units and measures should conform to the standards set out in Science 1954;120:1078.

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Front cover: A illustrates a collagenous enteritis due to olmesartan. Notice how IELs are not prominent in this case, an event occurring in a minority of cases. In B is Taxol effect in Barrett’s esophagus, a mitosis with a central bar in it is present in the center. C1 and C2 show mycophenolate colitis. In C1 eosinophilic exhausted epithelium stretches along a injured crypt. In C2 the appearance of the mucosa with crypts drop out, distortion and damaged crypts is present, page 105.
Melanocytic nevi
and non-neoplastic hyperpigmentations

C. CLEMENTE
Servizio di Anatomia Patologica e Citodiagnostica, I.R.C.C.S. Policlinico San Donato, Milano

Preface

This is the first of three chapters that will be progressively published on Pathologica as updating activity of the Italian Study Group of Dermatopathology (GISD), Italian Society of Pathology and Cytology (SIAPeC IAP). The first chapter concerns non-neoplastic hyperpigmented skin lesions and nevi, the second will address the topics of dysplastic nevus, borderline and low malignant potential melanocytic proliferations and the third melanoma in its variants and differential diagnoses with a supplement on the immunohistochemistry and molecular support to diagnostic and prognostic definition of nevi and melanomas. Although we believe that great advances were made in the application of ancillary genetic, immunohistochemical and molecular techniques, for the diagnosis and biological characterization of melanocytic tumors the morphology still remains the gold standard. These chapters are not intended as substitutes or even claim to be compared to the numerous and valuable texts that are also recently published, but they want to present, concisely and quickly available, all of those traits that we believe essential to the histopathological evaluation of a melanocytic lesion. No morphological parameter is exclusive and individually sufficient to make the correct diagnosis of nevus or melanoma but to reach a final conclusive and appropriate interpretation a set of morphological characters must be evaluated and compared. I was lucky enough to be able to examine several thousand cases and to draw lessons from each of these increasing my diagnostic experience. I had a great lesson by my teacher and good friend Prof. Martin C. Mihm Jr of Boston, dermato-pathologist with undisputed international reputation, who, with great passion, patience and friendship, transferred me much of his experience and knowledge and for which I always thank him. Special thanks I would like to address Dr. Agostino Crupi, dermatologist, skin-oncologist and brilliant dermatoscopist who taught me how the diagnosis of melanocytic lesions starts from the clinic examination and the mutual comparison between dermatologist and pathologist is a great richness of knowledge for both. Finally thank to my collaborators Barbara Rubino, Barbara Bruni and Antonella Festa for the large number of material collected in these years at the Pathology Service of the IRCCS Policlinico San Donato and a particular thank to Marco Turina who collaborated in the drafting of this text.

Milan, March 2017
Claudio Clemente

01 Non-neoplastic hyperpigmented lesions

01A Ephelides (freckles)

Ephelides are light tan, uniform skin lesions with irregular and poorly defined borders, frequently less than 2 mm in diameter, on light-exposed skin (particularly the face, dorsa of the hands, forearms and perioral skin), shoulders and upper limbs. Ephelides are more evident after sun exposure and they are inconspicuous in winter. They are numerous in people with fair skin, red hair and clear blue eyes. Ephelides are characterized by hyperpigmentation of the epidermal basal keratinocytes without melanocytic proliferation and elongation of rete ridges. The skin appendages are usually not involved. Pigmented melanophages may be dispersed in the superficial dermis.

The differential diagnoses include the lentigo simplex, characterized by slight or variable increased melanocytic

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proliferation with lentiginous elongation of the rete ridges, the café au lait spot that shows a moderate or slight increase of melanin pigment with giant melanosomes and normal epidermal architecture. Melanotic macules are benign, oral pigmented lesion more frequently in females, adulthood, usually in the lower lip (labial melanotic macule) and gingiva. Unlike an ephelides, the melanotic macules do not become darker with sun exposure. Other differential diagnoses are amalgam tattoo and focal traumatic ecchymosis, the last to correlate to anamnestic data.

<table>
<thead>
<tr>
<th>Ephelides: key histopathological diagnostic features</th>
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<tbody>
<tr>
<td>• Increased content of melanin in keratinocytes without evidence of melanocytes proliferation</td>
</tr>
<tr>
<td>• No rete ridges elongation and junctional nests</td>
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<tr>
<td>• Normal epidermal architecture</td>
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</table>

### 01B Actinic (solar) lentigo
Actinic (solar) lentigo are irregular and focal areas of hyperpigmentation of the skin more large (about 4 to 10 mm in diameter) than the ephelides, with a slight increase of the basal melanocytes number. Frequently the lesions are present in middle age, in individual with fair skin (photo-type I). Actinic (solar) lentigo represents a heterogeneous group of lesions characterized by different patterns that depends on the state of its evolution. In the early stage, there is uniform slight basilar hyper-melanosis and mild squamous atypia. In evolved stages, epidermis shows irregular lentiginous elongation of rete ridges with hyperpigmentation and increase of melanocytes. In the superficial papillary dermis may be present heavily pigmented melanophages, few sparse lymphocytes and variable elastosis frequently also severe. The single intraepidermal melanocytes are sometimes enlarged and immature but not organized in nests. The differential diagnoses include the hyperpigmented variant of actinic (solar) lentigo (reticulated lentigo, ink spot lentigo) that clinically may suggest a melanoma but atypia is light and focal, the basal proliferation of the melanocytes is not continuous and lack of aggressive epidermal infiltration with apoptosis and necrosis of the keratinocytes. In lentigo maligna a well evident a continuous basal proliferation of atypical melanocytes with extension to hair follicles is usually well evident with irregular junctional nesting and focal atypical cells migration into the spinous layer of the epidermis. Hyperplastic rete is present in actinic lentigo whereas lentigo maligna shows flattening of the rete ridges with atrophy and dermal elastosis. In the pigmented actinic keratosis the skin shows variable keratinocytic atypia extended to the whole thickness of the epidermis. The pigmented seborrhic keratosis is characterized by acantosis and increased proliferation of the supra-basal keratinocytes. In the large cell acanthoma the basal keratinocytes are uniformly enlarged with increase of the nuclear cytoplasmic ratio; the immunostaining with HMB45 (melanocytes positive and keratinocytes negative) may be useful in the differential diagnosis. Becker’s nevus (pigmented hairy epidermal nevus) in a rare hamartomatous lesion with acanthosis, hyperkeratosis and elongation of the rete ridges and marked hyperpigmentation of basal keratinocytes. Increased number of smooth muscle hamartomatous bundles are present.

### 01C Mucosal melanosis
Mucosal melanoses include different pigmented lesions: genital lentigines (vulvar, vaginal and penile melanosis), labial melanotic macule frequently on the lower lip or rarely localized in other areas of the oropharynx and conjunctive. Alternating irregular areas of pigmented and normal mucosa may simulate a melanoma with regression. The mucosal melanosis may be variable in color, diffusely dark brown and to measure up to 15 mm of diameter and sometimes, in genital areas, several centimeters. Histologically a diffuse basal layer hyperpig-
with lentiginous growth pattern and spread to the adnexa.

**Mucosal melanosis: key histopathological diagnostic features**
- Hyperpigmentation of basal keratinocytes
- Increased number of melanocytes with long dendrites through the inter-keratinocytes space to the superficial layers of the epidermis
- Absence of nested proliferation and cytologic atypia

**02 Acquired melanocytic nevi**

**02A LENTIGO SIMPLEX**

Lentigo simplex is a common melanocytic lesion localized anywhere in the tegument, not related to sun exposure. Clinically it is a well-circumscribed, uniformly pigmented, tan brown to black, 0.1-0.5 cm in maximum dimension. They are very common in childhood and adolescence. Histologically lentigo simplex shows an increased number of melanocytic cells in the dermo-epidermal junction with a slight elongation of the rete ridges. This lesion is not associated with nests of the nevus cells. There is an increased pigmentation both in the epidermal and in the papillary dermis within melanophages and sparse dermal chronic inflammatory infiltrate. When present in a large number they may be a feature of a syndrome (LEOPARD, LAMB, Carney’s complex, Peutz-Jeghers, Laugier-Hunziker). Occasionally small...
nest of melanocytes are present at the tip of the rete ridges and the term “jentigo” has been used (Ber Rahman and Bhawan 1969). A variant of the lentigo simplex is the nevus spilus which clinically presents small hyperpigmented macules and papules and histologically may be indistinguishable from lentigo simplex. The differential diagnoses include psoralen and ultraviolet A (PUVA) lentigo, a lesions with an irregular hyperplasia of the epidermis with scattered atypical junctional melanocytes, pigmentation of the basal keratinocytes extending to the granular and spinous layer with a like pagetoid spread. Others differential diagnoses include freckle, junctional nevus, or dysplastic nevus. The freckle is a not proliferative lesion of melanocytes associated to hyperpigmentation of the basal skin layer. If junctional nests are present in lentigo it is impossible to exclude that the lesion represents a true early junctional nevus. The dysplastic nevus presents a proliferation of nevus cells along the dermal/epidermal junction with lentiginous pattern and evident cytologic atypia; furthermore there are stromal changes including increased vascularity, prominent inflammation associated with striking lamellations of collagen. Senile lentigo shows irregular rete ridges hyperplasia with dense hyperpigmentation without melanocytic hyperplasia and frequently focal irregular melanocytic atypia. Acréal lentigo is a pigmented macula on the palm or soles and beneath the nails.

In malignant lentigo the prominent single cell proliferation along the epidermis may appear benign in early lesions and simulate a lentigo simplex. Malignant lentigo presents a strikingly atrophic epidermis, sun damaged dermis, and often a bandlike or lichenoid inflammatory infiltrate admixed with melanophages; furthermore the proliferation of melanocytes extends along the external root sheath of the hair follicles, even to the base of the hair follicle. Mucosal lentigines (labial and genital), vulvar, penile and acral lentigines may closely resemble lentigo simplex with increased number of intraepidermal basal pigmented melanocytes with well evident dendritic morphology.

<table>
<thead>
<tr>
<th>Lentigo simplex: Key histopathological diagnostic features</th>
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<tbody>
<tr>
<td>Hyperpigmentation of the basal layer of the epidermis</td>
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<tr>
<td>Lentiginous elongation of the rete ridges</td>
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<tr>
<td>Irregular increase of the number of junctional melanocytes</td>
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**02B JUNCTIONAL, COMPOUND AND DERMAL NEVI**

Melanocytic nevus is a benign tumor, most often found during childhood or adolescence, which may develop on hair bearing skin or also on other sites including glabrous skin, nails or mucosae. The most frequent sites in males are head, neck and trunk; in females lower limbs. The majority of nevi change and regress in the elderly. They are more common in individuals with pale skin (phototype 1). Three nevi types can be recognized: junctional, compound and dermal nevi. Clinically junctional nevi are macular, flat, small, up to 0.5 cm in greatest dimension, uniformly pigmented and well circumscribed. Color vary from light to dark brown. Compound nevus usually is usually raised, it can be dome-shaped, warty, and often darkly pigmented. Intradermal nevi often have scarce or no pigmentation, they can be pedunculated or papillomatous/dome-shaped.

The histological features of nevi depend upon the stage of maturation. Junctional nevi exhibit nests of nevomelanocytes in the basal and lower part of the epidermis, usually at the tips of the slightly hyperplastic rete ridges but they can present also in a lentiginous pattern. A nest is considered if five or more cells in a single cluster are present. The melanocytic cells are polygonal, epithelioid or spindled, with well-defined cell boundaries; the cytoplasm is clear or pale, eosinophilic to amphophilic, with sparse, usually coarse, melanin pigment. The junctional and superficial dermal melanocytes present nuclei round to oval and have prominent nucleoli (type A cells). The aggregates of the melanocytes are cohesive and a clear space separates the nest from the adjacent epidermis. To note the regular and repetitive nesting architecture. Occasionally the nevi of the childhood, nevi in pregnancy and in special sites as the scalp and genitalia, a junctional nevus may simulate an in situ or superficially invasive melanoma. Despite the architectural disorder the junctional melanocytic proliferation is usually monomorphic and discontinuous without pagetoid spread at the shoulder of the nevus. A variant of the junctional nevus, frequently observed in elderly patients, shows elongation of the rete ridges in lentiginous pattern.
granules, there is less retraction from keratinocytes of the nests and it is evident an “aggressive” proliferation with keratinocytes apoptosis. 

**Compound nevi** show junctional and dermal nests of nevus cell, extended to the papillary dermis in acquired nevi and to reticular dermis in congenital nevi. They are well-circumscribed and symmetrical lesions and usually do not have the “shoulder phenomenon”. In dermal component the cells are smaller, resemble lymphoid cell, have less cytoplasm and dark nuclei (type B cells) and in deep component the nevus cell are spindle, fibroblastic-like (type C cells). Mitotic activity is very low and atypical mitoses are absent.

A lentiginous proliferation, simulating a dysplastic nevus may be variably present in the compound congenital nevus, localized in the central area of the lesion. Usually the intraepidermal component does not extend beyond the dermal component but if present, the extent beyond the dermal component is symmetrical on both sides of the lesion. The junctional nests are identical to those described under the junctional nevus. Intraepidermal junctional nevus cells have been described by the designation type A epithelioid cells. These cells are large round nevus cells with coarse melanin granules within them. In

The differential diagnoses include the **pigmented junctional spindle cell nevus** composed of a uniform population of characteristic spindle cells in nests well defined and oriented perpendicular or parallel to skin surface. The **lentiginous junctional dysplastic nevus** that shows variation in morphology of the single cells and in the size of the nests. The dysplastic nevi are characteristically discohesive with proliferation along the lateral portion of the rete ridges not at the tip. **Superficial spreading melanoma** may show a prominent nesting pattern but the cells are large, have prominent eosinophilic nucleoli, large cytoplasm filled with fine, dust like, melanin

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Fig. 9a. Verrucous (Misher) nevus.

Fig. 9b. Dome shaped (Unna) nevus.

Fig. 10. Junctional nevus; nests of melanocytes at the tip of the rete ridges.

Fig. 11. Lentiginous proliferation in junctional nevus in elderly patients to differentiate from a lentiginous junctional dysplastic nevus.

Fig. 12. Compound nevus: superficially plump type A nevus cells and lymphoid-like type B cells at the middle and deep component are present. To note the different color of the dermal nevus due to crowding of nuclei and reduction of nuclear cytoplasmic ratio.
the dermal component of the lesion the picture is quite variable. In early lesion there are small nests of cells, many similar to type A cells, with large round nuclei present in the papillary dermis. These cells as they increase in number with lesion aging become smaller and round without pigment, type B cells. They exhibit tiny nucleoli and are associated with a fine fibroblast-like cells surrounding the nest. As the lesion ages, these cells are associated with a spindle shaped cell that has been designated a type C cell. The type C cells are associated with increase of the reticulum fibers separating the C cells from one another. The type C cells can become arranged in complex patterns resembling neurofibromatous. Type A cells express S100 protein and HMB-45. Type B cells may express either S100 or the Schwann cell associated antigen but they are mostly HMB-45 negative. This variation in morphology and immunohistochemical findings is considered as a maturation phenomenon of the nevus cells in their dermal component. Junctional and dermal nevus cells are usually p16 positive; the small B nevus cells may lost the p16 positivity. A variant of the compound nevus is considered the \textit{nevus spilus}, characterized by a junctional melanocytic hyperplasia of single cells or nest associated with a variable pigmentation of adjacent keratinocytes, elongation of rete ridges and thickening of papillary dermal collagen. In \textbf{differential diagnosis} the compound \textit{dysplastic nevus} exhibit an irregular proliferation of atypical nevomelanocytes in the epidermis overlying the dermal component. There is no symmetry in this lesion so that the dermal component lies eccentrically in relationship to the epidermal component. \textbf{In melanoma} in addition to irregular nesting intraepidermal pagetoid aggressive spread of malignant melanocytes is present at the shoulder of the lesion furthermore the characteristic maturation of type A to B to C cells is absent. In the vertical growth of a monomorphic, expansive, centrifugal proliferation is characteristic. The melanoma cells frequently exhibit finely granulated melanin pigment in their cytoplasm while it is an atypical and rare finding in compound nevi. Mitotic activity in the dermal component is extremely rare in compound and dermal nevi whereas it is common and variable in melanomas. The mitotic activity must be evaluated along all the proliferative margin of the lesion. Finally, compound nevi very rarely have a striking inflammatory response, whereas melanoma is commonly, especially below the intraepidermal component, associated with a lymphocytic host brisk response.

\textbf{Intradermal nevus} represents the late stage of maturation; it shows usually less pigmentation with progressive accumulation of fibrous stroma. The cells are frequently spindle with fibroblast or schwannian-like appearance (wavy nuclei, pale cytoplasm, fibrillar appearance, type C cells). The nevus cells are confined to the dermal component with no intraepidermal involvement. The epidermis may be flattened because of effacement of the rete ridges. The presence in the deep reticular dermis and around the adnexal structure suggests a congenital nevus. There is no evidence of prevalent expansile growth in the deep component of a dermal nevus. The dermal component shows a superficial deposition of melanin pigment and a proliferation of small nevomelanocytes between the dermal stroma.

\textbf{Fig. 13a. Intradermal nevus: there is not intraepidermal nevus. The dermal component shows a superficial deposition of melanin pigment and a proliferation of small nevomelanocytes between the dermal stroma.}

\textbf{Fig. 13b. Nevus with dermal nests.}

\begin{table}
\begin{tabular}{|c|}
\hline
\textbf{Junctional nevus: key histopathological diagnostic features} \\
\textit{Flat or slight raised pigmented lesion} \\
\hspace{1cm} Junctional nest of cohesive melanocytes \\
\hspace{1cm} Nests at the tip of the rete ridges \\
\hspace{1cm} Epithelioid or less often spindled morphology \\
\hspace{1cm} Uniform melanocytes in regular and repetitive nests \\
\hline
\end{tabular}
\end{table}

\begin{table}
\begin{tabular}{|c|}
\hline
\textbf{Compound nevus: key histopathological diagnostic features} \\
\hspace{1cm} Dome shape (Unna) or verrucous (Miescher) \\
\hspace{1cm} Circumscribed and symmetrical \\
\hspace{1cm} Junctional component usually not beyond the dermal component \\
\hspace{1cm} Maturation in depth \\
\hspace{1cm} Melanocytes type A (epithelioid), B (lymphocyte-like) and C (neural/fibroblastic like) \\
\hspace{1cm} Dermal mitoses rare or absent \\
\hline
\end{tabular}
\end{table}
Dermal nevus: key histopathological diagnostic features
- Dome shape (Unna) or verrucous (Miescher)
- Maturation with depth
- Type B and C cells
- Neurotization, multinucleate cells, pseudovascular and pseudoglandular change
- Stromal mucin deposits, fatty infiltration, sclerosis
- Pseudovascular invasion
- Dermal mitoses rare or absent

02C Halo nevus
Halo nevi, also termed leukoderma acquisitum centrifugum, are benign melanocytic nevi surrounded clinically by a hypopigmented zone. They usually occur in childhood or adolescence. The prevalence is about 1% of the population; its incidence in men and women is equal and the most frequent site is the trunk. In about half of the cases, multiple lesions can be observed. Vitiligo and halo nevi are strongly associated. It has been estimated to occur in up to 26% of patients which have vitiligo. The epidermis is often acanthotic and frequently hyperkeratotic, the nevus is usually compound and infiltrated by lymphocytes and histiocytes with occasional mast and plasma cells. The inflammatory infiltrate exhibits a band like extension throughout all the nevus, virtually obscuring the dermal component. The melanocytic junctional cells are organized in nests and clusters with rare or without pagetoid intraepithelial spread. The melanocytes have relatively large nuclei with small nucleoli and abundant eosinophilic cytoplasm, with focal signs of cellular atypia. The dermal melanocytic cells obscured by the inflammatory infiltrate may be highlighted using S-100 protein and thyrosinase. About 80% of the lymphoid cells are T-lymphocytes with a relatively high percentage of suppressor/cytotoxic T-cells. Epithelioid granulomata can occasionally be found within the inflammatory cell infiltrate. Frequently, pigment containing macrophages are found in the inflammatory cell infiltrate. There is no fibrosis, in contrast to melanoma. The halo nevus must be distinguished from regressing melanoma. An important feature to differentiate is the diffuse distribution of the inflammatory infiltrate throughout all the nevus while in melanoma usually the infiltrate is partial, multifocal and irregularly distributed. Mitotic activity and nuclear and cytoplasmic pleomorphism of the dermal component are not features coherent with halo nevus while are present in melanoma.

Halo nevus: key histopathological diagnostic features
- Presence of numerous lymphocytes in dermal and junctional component of the nevus
- Band like extension of the inflammatory infiltrate throughout all the nevus
- Junctional nevus cell with eosinophilic cytoplasm and focal atypia

02D Meyerson’s (inflamed) nevus
The Meyerson’s (inflamed) nevus is an eczematous process associated to a nevus. The eczematous lesion consists of psoriasiform and spongiotic epidermal changes with superficial perivascular dermal infiltrate. The nevus retains its characteristic features despite the inflammatory reaction. The Meyerson’s nevus must be differentiated from the halo nevus that represents an active lymphocyte-nevus cell response and that usually it is not associate with an eczematous process.

Balloon cell nevus
Balloon cell nevus is a benign melanocytic lesion most often diagnosed in the first three decades of life and with predilection for the head, neck and trunk. It can found also in other sites including iris, conjunctiva, caruncle, soft palate, pharynx. Clinically it presents as a red or brown papule or nodule, with no special features. Balloon cell nevus is composed of round to oval sized melanocytic cells with abundant foamy pale-staining or clear cytoplasm; it can be compound or entirely intradermal. The cell nucleus is hyperchromatic or vesicular, the nucleolus is conspicuous. Mitoses are rare or absent.
abdomen. The interval of time between the excision of the primary lesion and the recurrence is mostly about 3-6 months. The majority of cases occur in the first four decades of life. Clinically, recurrent nevi are usually flat and variously pigmented; most often stippled, but some are uniformly black, or irregularly pigmented. They may have different patterns including hypopigmentation, linear, halo, diffuse. They are usually less than 1.5 cm in diameter, with a variable degree of scarring, sometimes hypertrophic. They most frequently follow after shave biopsies, traumas, laser treatment, local application of topical agents. Recurrent nevi histologically show sharp circumscription, atypical melanocytes frequently confined to the epidermis, increased number of melanocytes, isolated or in nests within and above the basal cell layer. A great variation in the size and shape of melanocytic nests frequently confluent. Few atypical melanocytes with large hyperchromatic and pleomorphic nuclei and cytoplasmic coarse pigmented granules are present. The papillary dermis shows fibrosis and a perivascular sparse lympho-histiocytic infiltrate. Epidermal hyperplasia in a retiform pattern confined to the area of the scar can be seen. Some pagetoid spread sometimes is observed above the dermal scar and usually rete ridges is effaced above the fibrosis. Dermal melanophages are frequently conspicuous. Mitotic figures are rare or absent and apoptosis is not an evident feature. The main differential diagnosis is with superficial spreading melanoma and the clinical informations are mandatory. Histological points of distinction include: the restriction of the junctional component to the area overlying the fibrosis, sharp circumscription of the intraepidermal melanocytic component, atypical melanocytes confined to the epidermis, few, if any, mitosis, fibrosis in the papillary dermis.

Foci of balloon cells can found in 2% of different nevi and in melanoma. To identify this lesion as balloon nevus this cytoplasmic change must be present in over 50% of the cells. Balloon cells are positive for melanocytic markers but interestingly, a misleading CD68 positive balloon cell nevus on the penis in a child has been described. The most important differential diagnosis is with balloon cell melanoma. Important features to consider include age of the patient, cytological features like striking pleomorphism and large nuclei typical of malignancy, presence of mitoses and of an inflammatory infiltrate between the melanocytic cells. Other lesions may be similar to balloon cell nevus, including clear cell dermatofibroma, granular cell tumor, xantoma, sebaceous tumors, clear cell sarcoma, clear cell renal carcinoma metastatic to the skin.

Balloon cell nevus: key histopathological diagnostic features
- Presence of large cells with abundant vacuolated cytoplasm in number greater than 50%
- No mitoses
- Nuclear cytoplasmic ratio quite small

02F Recurrent (persistent) nevus
Recurrent (persistent) nevus is the development of a melanocytic lesion at the site of removal of a previous benign nevus. Its recognition is very important because it can resemble a regressing melanoma. There have a female predominance; mostly found on the back and abdomen.
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A immunohistochemical staining is also helpful to differentiate neurotized melanocytic nevi (positive) from neurofibroma (negative).

**Neuro nevus: key histopathological diagnostic features**
- Spindle cells similar to Schwann cells
- Mature collagen fibers
- Frequent presence of residual foci of common nevus
- No infiltration of adventitial dermis

**02H Aging (ancient) nevus**
Aging of dermal nevi has been associated with senescent changes as balloon cell changes, sclerosis, giant cells, mucinous degeneration, neurotization, and infiltration by fat cells. Ancient nevus, described by Kerl (1998), is a nevus generally localized on the face that presents a mixture of pleomorphic epithelioid melanocytes and small, uniform nevus cells, fibrosis, mucinosis, hemorrhage and vascular thrombosis.

**Ancient nevus: key histopathological diagnostic features**
- Balloon cell changes, sclerosis, giant cells, mucinous degeneration, neurotization, and infiltration by fat cells
- A mixture of pleomorphic epithelioid melanocytes and small, uniform nevus cells, fibrosis, mucinosis, hemorrhage and vascular thrombosis

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**02G Nevus with neurotization**
Melanocytes can mature into cells similar to Schwann cells characterized by spindled or S shape and surrounded by mature collagen fibers. The cells of the neurotized nevus can be organized in tactoid bodies or corpuscles similar to Meissner bodies or in a palisaded pattern. To differentiate neurotized nevi from neurofibromas one must observe the adventitial dermis of hair follicles and vessels. Neurofibromas infiltrate the adventitial dermis up the basement membrane of the external root sheath while neuro nevi respect the adventitial dermis. Melanocytes can mature into cells similar to Schwann cells characterized by spindled or S shape and surrounded by mature collagen fibers. The cells of the neurotized nevus can be organized in tactoid bodies or corpuscles similar to Meissner bodies or in a palisaded pattern. To differentiate neurotized nevi from neurofibromas one must observe the adventitial dermis of hair follicles and vessels. Neurofibromas infiltrate the adventitial dermis up the basement membrane of the external root sheath while neuro nevi respect the adventitial dermis.
02I Combined nevus
A combined nevus is the presence of two or more types of different nevi in the same lesion. The main component is usually an intradermal or compound nevus. The most frequent combinations are a common nevus with a blue nevus and a Spitz nevus, deep penetrating nevus combined with a blue nevus. The presence of foci of A/B melanocytic cells in deep dermis may simulate absence of maturation.

Fig. 20a. Combined nevus: compound and deep penetrating nevus.

Fig. 20b. Combined nevus: dermal and blue epithelioid nevus.

Combined nevus: key histopathological diagnostic features
- Well identified different common nevi
- No cytological atypia, mitoses and aggressive infiltrative growth
- Simulation of deep maturation

02K Other rare variants of nevi
Nevus of Nanta (dermal nevus with ossification)
Foci of ossification are present in the dermal component of a nevus, usually on the face of a woman, as the result of a folliculitis (Colin 2002) or result of a metaplastic process. Around the foci of ossification a rim of osteoblasts is usually present. Ossification may be observed also in Spitz nevus and in melanoma.

Elastic fibers in nevi
Frequently some increase of dermal elastin is present in intradermal, compound and blue nevi, especially at the periphery. In 0.5% of nevi there is an evident increase of coarse elastin fibers. This increase is most prominent in congenital nevi. These fibers are notably absent in melanoma and its presence may be useful to support the benign nature of a lesion.

Nevus with trichostasis spinulosa
This is a nevus, usually on the face, with associated dilated large hair follicles like comedones and numerous vellus hairs.

Fig. 21. Focus of metaplastic ossification in dermal nevus.

Fig. 22. Dermal elastin fibers, coarse and irregularly distributed between the dermal melanocytes.

Fig. 23. Dermal nevus associated to numerous vellus hairs.
Nevus with pseudo-vascular lacunae
Occasionally a compound or intradermal nevus contain irregular interconnected spaces empty or containing mucina, slit-like clefts with pseudo-vascular sinusoidal appearance. Pseudo-vascular spaces within the nevus cells give an angiomatosus-like appearance and protrusion of sub-endothelial nevus cells in vascular lumen may simulate vascular invasion. Staining with S100 protein, p16 and CD31 is useful to identify the nevus cells under the endothelial layer.

![Fig. 24. Dermal nevus (left), pseudo-vascular lacunae and nidus of sub-endothelial nevus cells (right).](image)

Nevus in combination with other not melanocytic neoplasms
Desmoplastic trichoepithelioma, basal and squamous cell carcinoma, syringoma, dermatofibroma, atypical fibroxanthoma, angioma, inclusion cysts may be associated with nevi. This combination is the result of coincidence not etiologically related.

![Fig. 25a. Dermal nevus and angioma.](image)

![Fig. 25b. Compound nevus and desmoplastic trichoepithelioma.](image)

Nevus with myxoid change
Mucin is occasionally present in nevi, preferably in the superficial portion of the dermal component. The mucin is often visible with hematoxylin and eosin stain or enhanced with alcian blue. Mucin deposits can be also present in Spitz nevus and in cellular blue nevus (myxoid blue nevus).

Nevus with amyloid
Amyloid deposits were identified within the tumour mass of three melanocytic naevi. Amyloid in this situation is likely to be derived from degenerating naevis cells.

Nevus with pagetoid cells
The presence of solitary and small groups of pagetoid melanocytes in the superficial layers of the epidermis it is generally considered to be a diagnostic hallmark of melanoma but it may also be seen in certain melanocytic naevi (nevus in special site, acral nevus, nevi of the scalp, Spitz nevi, nevi in childhood, Reed nevus, congenital nevi, nails nevi, genitalia nevi, flexures nevi, milk line nevi). In naevi, pagetoid intraepidermal spread tends to be limited laterally not beyond the underlying junctional component and there is no marked cytological atypia.

![Fig. 26. Traumatized nevus; skin ulcer and cap of fibrinoid inflammatory essudate.](image)

![Fig. 27. Acral nevus with intraepidermal pagetoid spread simulating a dysplastic nevus.](image)

Traumatized nevus
Nevi can be ulcerated, abraded and present irregular scar. Traumatized nevi have special features like parakeratosis, dermal telangiectasias, inflammation and fibrosis. These patterns may simulate a regressing melanoma.
**Lobulated intradermal nevus**
Lobulated intradermal nevus is described as an unusual sign of aging or regressing melanocytic nevus. The nevus clinically shows lobulation and histologically exhibits fat cell within nests of nevus cells, fibrosis and neurotization. In nevi, pagetoid spread tends to be limited and there is no marked cytological atypia and laterally beyond the underlying junctional component.

**Inverted type A nevus**
Nevus with multiple small nets of large pigmented A cell in the dermal component associated to a prevalent proliferation of lymphocyte like B cells and shwannian like C melanocytes. Some authors consider this variant a combined nevus (common nevus associated with deep penetrating nevus or epithelioid blue nevi).

**Nevus in pregnancy or hormonal therapy**
Uniform darkening and enlargement of nevi is common in pregnancy. Possible changes are mild degree of cytological atypia, active junctional proliferation and rare mitoses.

**Nevus in childhood**
Nevi in childhood are more proliferating and cellular compared with the nevi of adults. The nevus cells are larger in children than in adults and the deep maturation less evident. Mitoses may be seen, but atypical mitoses are absent. The trans-epidermal elimination of single or nests of melanocytes are not infrequent observation simulating an intraepidermal pagetoid spread.

**Incidental nevus**
Small nevi or aggregates of nevus cells are present in the dermis of skin excised for unrelated reasons. Frequently, in the face, these nevi have a peri-appendageal localization.

**Fat cells in intradermal nevus**
Aggregates of adipocytes within a dermal nevus mixed to type C spindle cell nevus cells. This is a pathway of regression and disappearance of the nevus; the other manners in which nevus cells disappear spontaneously are: halo reaction, neurotization and fibrosis.

**Nevus cells in lymph nodes (nodal nevus)**
Aggregates of nevus cells are occasionally observed within lymph nodes, usually small foci or isolated cells but rarely they may occupy most of the lymph node. The prevalence of nevus cells in the lymph nodes varies from 1% in patients undergoing surgery for breast cancer to 3.9% in patients with melanoma submitted to sentinel lymph node biopsy. Nodal nevus cells are often located in the fibrous capsule and trabeculae of the lymph node arranged in nests and strands. The nevus cells are small, cytologically benign with round or oval nuclei with delicate chromatin. Nodal nevus cells are negative with HMB-45 and positive with p16, S-100 and thyrosinase. Presence of capsular nevus cells are significantly associated with an increasing nevi count on the skin of the patients.
be junctional, compound or dermal. The main feature is characterized by a florid junctional melanocytic proliferation of large and variable sized nests often with retraction artifacts and/or cellular dis cohesion. Cytologic atypia, frequently present, vary from mild, to moderate and severe. The atypical melanocytes are frequently epithelioid, type A, with abundant eosinophilic cytoplasm and variably prominent nucleoli or polygonal with angulated cells with hyperchromatic nuclei and scant cytoplasm. Rare dermal mitoses and focal pagetoid intraepidermal spread, centrally located and usually confined to the stratum sub-corneum, can be seen. A subset of genital nevi show distinct features which may have some similarities with melanoma. This variant is defined atypical genital nevus that are found generally in young/adult female patients, with a mean size of 0.6 cm in diameter. Some “dysplastic features” can be seen in atypical genital nevi: focal bridging of rete ridges, a lentiginous single cell proliferation, dusty cytoplasmic melanin, mild superficial chronic inflammatory infiltrate and dermal fibrosis.

Despite occasionally striking cytologic and architectural atypia the atypical genital nevi have a benign clinical course. The recognition of these melanocytic lesions is important to avoid over diagnosis of melanoma. The main differential diagnoses are with dysplastic nevus in which the lentiginous proliferation is disorganized, rete ridges are elongated and cytological atypia is focal and random whereas in genital nevus and in atypical genital nevus it is relatively uniform. Melanoma shows a predominance of single pagetoid melanocytes compared

03 Nevi of special sites

Genitalia, acral skin, milk line, umbilicus, ear, scalp and body folds are considered special sites. The majority of nevi in special site are compound nevi and are characterized by large and confluent junctional nests sometimes with bizarre shape and variable cytologic atypia. The melanocytic lesions in flexural sites may have identical worrisome histological features.

03A Genital nevi

Melanocytic lesions of the genital area are rare. Anatomic sites include the labium major, mons pubis, labium minus, clitoris and perineum. The genital nevi can
to nests, greater cytological atypia and dermal mitoses, lack of deep maturation and generally it occurs in postmenopausal women.

**Genital nevi: key histopathological diagnostic features**
- Single cells and irregular confluent nests at the dermal epidermal junction
- Uniform cytological atypia
- Lentiginous proliferation with fusion of the nests
- Dyscohesive nests with retraction artifact
- Fibrosis in papillary dermis

**03B Acral nevus**

Acral nevus is a benign melanocytic lesion which is found on the palms and soles of hands and feet. They are found in approximately 25% of the entire population, with no difference of prevalence between men/women and whites/negroes. Peak of incidence is between second and third decades, and rarely acral nevi are found in infants. The clinical diameter ranges from 0.5 mm to 12 mm, with a median of 2 mm. They are usually symmetrical and well circumscribed, uniformly pigmented, as dark brown to black macule/papule. The main features of acral nevi are circumscription, symmetry, and some degree of fibroplasia. The melanocytic nests are variable in size, frequently vertically oriented, and they are found along the dermo-epidermal junction as a lentiginous and quite continuous proliferation. Mild to moderate pagetoid spread is commonly found, and if it is very marked, the name MANIAC (melanocytic acral nevus with ascent of cells) have been used to classify the lesion with this feature. Other typical features of acral nevi are: trans-epidermal elimination of nests, maturation of the dermal component, and no mitotic activity. The main differential diagnosis is with dysplastic nevus, which shows the shoulder phenomenon, dusty pigmentation, and bridging, in addition to lamellar and eosinophilic fibrosis. To differentiate acral nevus from dysplastic nevus and melanoma Clemente et al. described a special variant named acral lentiginous nevus. This variant is characterized by lentiginous melanocytic proliferation with confluent nests at the dermal epidermal junction, dermal fibrosis and cytological atypia. Acral lentiginous melanoma usually shows irregular epidermal acanthosis, severe cytological atypia, mitotic activity, no maturation of the dermal component and often a dermal irregular lymphocytic infiltrate and destructive intra-epidermal growth.

**04 Spindle and epithelioid cell nevi**

**04A Spindle and epithelioid cell nevus (Spitz nevus)**

Spitz nevus is a benign, infrequent, melanocytic tumor with a wide range of distribution but it occurs mainly in childhood and in adolescence. The most frequent locations are head and neck, especially the cheek, extremities, in a female, and trunk. Its male to female ratio changes according to the age of presentation: 1/1 between 0-15 years and beyond 45; 3.5/1 and 1.9/1 in patients respectively between 16-30 years and 31-45 years. The mean age of presentation is 21 years old (range: 2 to 69). It is very rare to encounter in black patients and oriental. Clinically it usually appears as asymptomatic, dome-shaped, round to oval, firm papule or nodule. The Spitz nevi are pink, red or tan, sometimes described as nonpigmented or purple, brown/black in differential diagnosis with dermatofibroma and hemangioma. Spitz nevi can range in size from 1 mm to 3 cm; most are less than 1 cm in diameter. Ulceration is usually absent or either rarely seen. Spitz nevus may be compound, junctional or entirely intradermal. The classical compound tumor is dome-shaped, with lateral circumscription and symmetry. Spitz nevi has two types of cell components: spindle, epithelioid or mixed.
The epidermis frequently shows hyperkeratosis, patchy parakeratosis, acanthosis, sometimes marked pseudo-epitheliomatous hyperplasia. Kamino bodies are found more often in junctional/compound Spitz nevi than in intradermal nevi; often in aggregates, they are situated at the dermo-epidermal junction. The classical epithelioid Spitz cells show a dense eosinophilic cytoplasm, elongated, ovoid or round nuclei with a well demarcated nuclear membrane and a prominent eosinophilic nucleolus. Maturation is seen towards the dermal component of the lesion and it is characterized by cells with scanty cytoplasm that have a single-cell infiltrating pattern at the base of the tumor. Sometimes, in children, the maturation is limited to small number of cells to demonstrate with immunohistochemical stains. Mitoses can be found in the nests located in the epidermis or superficial dermis and they may even be quite common in lesions in infants or young children. Very rarely or never the mitoses are atypical. A perivascular, diffuse or band-like inflammatory infiltrate can be found in up to 70% of cases, ranging from light, to moderate/severe. Telangiectasias are found in about half of the cases, more commonly in compound and intradermal Spitz nevi in the upper part of the papillary dermis. Melanin is found in about 50% of cases, more often in junctional/compound nevi than in intradermal nevi; it ranges from scarce, to abundant and rarely heavy. Spitz nevus shows strong and diffuse S100 protein expression, both in junctional and in dermal components. HMB-45 usually shows and irregular patchy positivity; p16 is often diffusely positive both in

<table>
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<th>Tab. I. Variants of Spitz nevus.</th>
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<tr>
<td><strong>Angiomatoid</strong> (Diaz-Cascajo C Am J Dermatopath 2000; Kwun O Ann Dermatol (Seoul) 2008; Rawson R Pathology 2016;48:739-42)</td>
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<tr>
<td><strong>Agminated</strong> (Gupta R Indian J Dermatol 2015)</td>
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<td><strong>Combined</strong> (Lin ZH J Dermatol 2000)</td>
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<td><strong>Desmoplastic</strong> (Barr R Cancer 1980; Dhouib RS 2008; Nojavan 2009)</td>
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<td><strong>Epithelioid cell of eyelid</strong> (Hiscoct P Am J Ophthalmol 1998;126:795-7)</td>
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<td><strong>Halo</strong> (Fernandez-Flores A Am J Dermatopathol 2017;39:120-3)</td>
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<td><strong>Intradermal</strong> (Plaza J Am J Dermatopathol 2014;36:283-94)</td>
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<td><strong>Lichenoid</strong> (Harrel JD J Cutan Pathol, 1997)</td>
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<td><strong>Lipoblastoid/signet-ring cell-rich</strong> (Savaito T J Cutan Pathol 2014;41:672-4)</td>
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<tr>
<td><strong>Myxoid</strong> (Hoang MP J Cutan Pathol 2003)</td>
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<tr>
<td><strong>Multiple</strong> (Zayour M J Am Acad Dermatol 2012;67:451-8)</td>
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<tr>
<td><strong>Oral cavity</strong> (Pediatr Dermatol 2016;33:e154-5)</td>
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<tr>
<td><strong>Pigmented epithelioid cell nevus</strong> (Choi JH J Am Acad Dermatol 1993;28:497-8)</td>
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<tr>
<td><strong>Polyoid</strong> (Fabi G Br J Surg Pathol 2000;142:128-32)</td>
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<tr>
<td><strong>Pseudogranulomatous Spitz nevus</strong> (Marco VS J Cutaneous Pathology 2012)</td>
</tr>
<tr>
<td><strong>Rich in melanophages</strong> (Cellenlo I Acta Derm Venereol 2002)</td>
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Epithelioid and multinucleated cells are sometimes found. The spindle cells have slightly eosinophilic or amphophilic cytoplasm with abundant melanin pigment. At the dermal-epidermal junction the proliferation of the tumor cells is discontinuous. The dermal component is usually inconspicuous and consists of small fascicles of spindle cells and single cells, exhibiting maturation with smaller nuclei and cell masses. The cytoplasmic melanin is present to a varying degree, from moderate to heavy in the majority of cases and nucleoli are prominent. Mitoses may be present, usually rare. The trans-epidermal migration of single cells or nests is frequent and it should not be misinterpreted as aggressive pagetoid invasion of the epidermis.

**Spitz nevus: key histopathological diagnostic features**
- Symmetric papule or nodule that involve papillary and reticular dermis
- Hyperkeratosis and epidermal hyperplasia
- Uniform cytology from side to side
- Maturation in deep component of the lesion
- Sharp demarcation of the lateral borders

**04B Pigmented Spindle Cell Nevus (Reed Nevus)**
Reed nevus is benign melanocytic lesion which it can sometimes be misdiagnosed, clinically and histologically, as melanoma. Clinically it presents as a usually recent and rapid onset heavily pigmented papule. Usually it is less than 10 mm in diameter, with a mean size of a 4.5 mm. It occurs frequently on the lower limbs or trunk of a female patient in the second/third decade of life (median age 25). Histologically Reed nevus has a fairly well marked lateral symmetry, sharp demarcation, usually with defined theques of junctional spindle cells. The overlying epidermis often shows some degree of hyperplasia, and in a minority of cases single melanocytes at the lateral epidermo-dermal junction. The tumor is composed by large and orderly arranged fascicles of spindle cells confined to the epidermis and papillary dermis differently oriented, parallel or perpendicular to the epidermis surface.

Epithelioid and multinucleated cells are sometimes found. The spindle cells have slightly eosinophilic or amphophilic cytoplasm with abundant melanin pigment. At the dermal-epidermal junction the proliferation of the tumor cells is discontinuous. The dermal component is usually inconspicuous and consist of small fascicles of spindle cells and single cells, exhibiting maturation with smaller nuclei and cell masses. The cytoplasmic melanin is present to a varying degree, from moderate to heavy in the majority of cases and nucleoli are prominent. Mitoses may be present, usually rare. The trans-epidermal migration of single cells or nest is frequent and it should not be misinterpreted as aggressive pagetoid invasion of the epidermis.
Eosinophilic globules (Kamino’s bodies) are present in up to 80% of cases. A frequently perivascular host inflammatory cell response, made by lymphocytes and melanophages, are seen at the deep lower margin of the lesion. Rare variants of Reed nevus are hypopigmented Reed nevus which shows all the typical features of conventional pigmented spindle cell nevus, but it does not contain abundant melanin and the mainly epithelioid Reed nevus. HMB45 immunohistochemical stain is positive in the intraepidermal and junctional component of Reed nevus and spindle cell melanoma, whereas dermal component is negative in pigmented spindle cell nevus while it is irregularly positive in spindle cell melanoma. The main differential diagnosis is with melanoma.

Reed nevus: key histopathological diagnostic features
- Circumscribed and symmetrical
- Junctional or compound
- Oval expansile junctional nests parallel or perpendicular to skin surface oriented
- Spindle uniform nevus cell with small nucleoli
- Junctional mitoses not uncommon
- Heavy pigmentation common
- Single cells or small intraepidermal nests (trans-epidermal migration)
- No pagetoid spread at the shoulders

05 Dermal melanocytosis

Dermal melanocytosis is a group of melanocytic lesions with dendritic fine pigmented melanocytes, oval or round nuclei and tiny nucleoli. A common immune-histochemical feature is the diffuse and intense immunohistochemical HMB-45 positivity. The group of dermal melanocytosis includes: blue nevus, cellular blue nevus, desmoplastic nevus, deep penetrating nevus, Mongolian spot, nevus of Ota and Ito and neurocristic hamartoma.

05A Blue nevus

Blue nevus is a common heavily pigmented lesion of bipolar and dendritic dermal melanocytes. The female to male ratio is 2.5 to 1; range of presentation is 1 to 79 years, with an average of 38.6 years, and a peak of incidence between 30 and 39 years. It can be found both in Caucasian and in Negroes. The topographic distribution in a decreasing order of frequency is: scalp and face, hand, arm and forearm, foot, buttock and sacro-coccygeal area, chest and breast. Blue nevus usually occurs in skin, but it has been reported in oral mucosa, sclera, uterine cervix, vagina, prostate, spermatic cord, pulmonary hilus, orbit, conjunctiva, maxillary sinus, lymph nodes. Clinically, blue nevus presents as a flat or discretely raised, dome-shaped, lesion. Its largest diameter is between 0.3 to 0.9 cm and the color varies from blue, to blue-black and dark brown. Blue nevus is predominantly situated into the dermis and sometimes can extend to the subcutaneous fat. Usually junctional activity is not seen, unless there is a not infrequent combined blue nevus. It presents two components: melanocytes and melanophages with stromal reaction. Melanocytes are spindle-shaped, bipolar, with dendritic processes and cytoplasm stippled with fine melanin granules, frequently grouped in multiple irregular bundles in the dermis usually parallel to the epidermis.

The dermal component can shows a marked desmoplastic reaction and numerous melanophages. Blue nevus usually follows skin appendages, blood vessels and nerves. Mitotic figures are rarely found. Some cases with satellitosis mimicking melanoma have been reported. Variants of classical blue nevus have been described: the most frequently encountered are epithelioid, desmoplastic, plaque types and blue nevus with ipercellularity.

An intermediate entity is the blue nevus with ipercellularity in which the cellular proliferation is marked but it
is absent the biphasic pattern characteristic of the cellular blue nevus. The epithelioid blue nevus may be sporadic and it is mostly associated with the Carney complex (autosomal dominant condition characterized by cutaneous lentigines, mammary, cutaneous and cardiac myxomas, Cushing’s syndrome, acromegaly, and sexual precocity). The epithelioid blue nevus is often part of a combined nevus. The blue nevus tumor cells are positive for melanin stains, S-100 protein and all other melanocytic markers and particularly it presents a diffuse and marked HMB-45 positivity. Blue nevus must be differentiated from desmoplastic melanoma which shows an atypical lentiginous melanocytic proliferation, mitoses also atypical mitoses, microscopic perineural or endoneural involvement, expression of S-100 protein but negative p16 stain.

**05B Cellular blue nevus**

Cellular blue nevus is an uncommon benign melanocytic lesion which occur more often in females. The female/male ratio is 2.2:1. It can present at all ages, but, coarsely, the peak of incidence at presentation is between 10-40 years old (Rodriguez et al reported a range of 7-60) and a mean age of 32.6 years. Occasionally it occurs at birth. It is more frequent in Caucasians than in Negroes. About 50% of cellular blue are located over the skin of buttocks and sacro-coccygeal region, then on the scalp and face. It can also occur on other different sites which include foot, hand, arm and forearm, knee, chest and breast. Cellular blue nevi have been also described in other locations including spermatic cord, female genital
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motic melanocytes and melanophages. Cellular atypia is infrequent; occasionally, multinucleated cells are seen. Cellular blue nevus is devoid of any type of inflammatory response within the dermal component and/or at the periphery; necrosis is absent unless associated with hemorrhage. Mitotic activity is almost absent or less than 1 per mm/2. Melanogenesis is variable: in some cellular blue nevi the tumor cells are heavily pigmented, in others a silver stain is required to demonstrate melanin. A fascicular or neuronevoid variant is rarely seen: it consists of fascicles and nests of spindled amelanotic melanocytes with clear cytoplasm, surrounded by fibrous tissue with numerous melanophages and dendritic melanocytes. Other variants of cellular blue nevus have been described: a sclerosing one simulating melanoma, balloon cell, amelanotic, angiomatoid, ancient and desmoplastic. Cellular blue tumor cells show uniform and strong positivity for HMB-45 antibody. A small subset of are CD34 positive, appearing to fit within the spectrum of neurocristic cutaneous hamartomas and to arise from more primitive neurocristically derived cells. Gerami et al. showed 100% sensibility and specificity in distinguishing cellular blue nevi from blue nevus-like melanoma, using an assay targeting 6p25 (RREB1), 6q23 (MYB), 11q13 (CCND1) and the centromere of chromosome 6 (Cep6) of fluorescence in situ hybridization technique (FISH). The main differential diagnosis is with malignant blue nevus/blue-nevus-like melanoma: the features which favor malignancy include: tumor size greater than 1-2 cm, infiltrative borders, necrosis, pleomorphism, high mitotic rate and atypical mitoses.

**Cellular blue nevus: key histopathological diagnostic features**
- Well circumscribed dermal nodule
- Extension to subcutaneous fat with a dumbbell pattern
- Nested and biphasic growth
- Clear and bipolar dendritic cells
- Mitoses rare or absent
- Vascular ectasia, hemorrhagic foci, myxoid change, cyst formations
- Necrosis absent
- Neural infiltration

**05C Desmoplastic nevus**

Desmoplastic nevus is a rare, benign melanocytic lesion with a female predominance. The age of presentation is wide, but most patients are in third decade. It predominantly affects the trunk, extremities, and the face. Desmoplastic nevus usually presents as an erythematous or red-brown papule or nodule, which can resemble intradermal nevus, atypical nevus, melanoma, and/or pigmented basal cell carcinoma. It is generally small, with an average of 3.5 mm in diameter, symmetric and well-circumscribed. At scanning magnification, the lesion has a symmetric microscopic growth pattern, it can be compound or entirely situated into the papillary and often extended to reticular dermis. Desmoplastic nevus mainly presents two components: melanocytic spindle or Spitzoid cells and a very dense stroma. It is unclear
whether desmoplastic nevus is a variant of Spitz nevus, sclerosing blue nevus, deep penetrating nevus with spindle type C cells or a distinct entity. It can show junctional activity characterized by the presence of both nested and lentiginous melanocytic proliferation or it may be unaffected. In the dermis, tumor cells are pleomorphic, and rarely have Spitzoid features especially in the superficial dermis; they have abundant eosinophilic cytoplasm, dark or vesicular nuclei and vesicular nucleoli. Intranuclear cytoplasmic pseudoinclusions (cytoplasmic invaginations) are often prominent.

Desmoplastic melanoma is the main differential diagnosis: morphologically melanoma has an in situ component and a dermal component made by spindled cells with nuclear basophilia and hyperchromatism, deeper invasive growth pattern, frequent perineural invasion and patchy lymphoid aggregates. Desmoplastic nevus shows low proliferative index assessed by mitotic count and/or ki67 expression while in desmoplastic melanoma mitoses, also atypical, are present. By immunohistochemistry, virtually all desmoplastic nevi are HMB-45, Melan A and p16 positive and desmoplastic melanomas are almost totally negative for HMB-45, positive in only 3% of cases for Melan-A and in 18% they present a focally and weakly p16 positivity.

Desmoplastic nevus: Key histopathological diagnostic features
- Spindle cell proliferation
- Extension to deep reticular dermis and subcutaneous fat
- Small spindle cells with scant cytoplasm
- The cells are intermingled with dermal collagen fibers
- Extension along the neurovascular bundles

Deep penetrating nevus is a benign melanocytic lesion which has an age of presentation quite large: 3-64 years, very rare after sixth decade, with the peak between second and third decades of life. It usually presents as a solitary nodule or papule of less than 1 cm in diameter. A slight female predominance (female to male ratio of 1.3:1) is reported. Most lesions are darkly pigmented but pigmentation can be variegated, from light brown to black, especially if part of a combined melanocytic nevus. The lesion may appear asymmetrical and unevenly pigmented, thus raising a clinician suspicion of melanoma. The most frequent site is the skin of the head and neck, followed by the extremities and trunk; Robson et al. describe only a deep penetrating nevus on the toe of a 10yo male, demonstrating the rarity of this tumor in acral sites. At scanning magnification, deep penetrating nevus has a sharply demarcated, circumscribed, often symmetrical and usually wedge-shaped configuration. The base is toward the epidermis and the tip toward the reticular dermis/subcutis with a narrow portion and frequent extensions along the skin adnexa or neurovascular bundles. The epidermis may show junctional melanocytic hyperplasia or it may present a limited junctional melanocytic component in from 60% to 85% of cases. Focal upward epidermal migration is rarely found and it should be seen carefully to exclude melanoma. The papillary dermis is frequently absent. The stroma is dense and sclerotic dermatofibroma-like, made by eosinophilic collagen bundles, or fibrillar and neurofibroma-like. The tumor cells are S-100 and HMB-45 positive and have moderately to strong nuclear positivity for p16. In differential diagnosis, epithelioid benign fibrous histiocytoma presents usually with an epidermal collarette and the tumor cells are round, with abundant eosinophilic cytoplasm and a vesicular nucleus with small eosinophilic nucleoli. By immunohistochemistry, fibrous histiocytoma shows positivity for CD34 and factor XIIIa and negativity for S-100 and Melan-A.
MELANOCYTIC NEVI AND NON-NEOPLASTIC HYPERPIGMENTATIONS

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and usually predominates while the spindle-cell morphology may be found in the deeper parts. In the deep portion, individual melanocytes appear to lie singularly between the connective tissue bundles, with frequent evidence of cytological atypia, suggesting an erroneous consideration of malignant lesion.

Fig. 45a. Deep penetrating nevus; extension of the nevus proliferation along an hair follicle into the dermis without evidence of progressive maturation of the nevus cells.

Fig. 45b. Deep penetrating nevus; spread of the pigmented cell between the collagen fibres as small nodules.

Fig. 45c. Deep penetrating nevus; the cells are rather large oval or spindle shape with amphophilic cytoplasm containing dusty pigment. The nest of melanocytes are surrounded by pigmented melanophages.

The melanocytic tumor cells have moderate to abundant cytoplasm, lightly to moderately pigmented, with often areas of clear changes. Nuclei are hyperchromatic, with variation of their size and shape from round to oval, mild to moderate nuclear pleomorphism, and no obvious maturation of melanocytes. Cytoplasmic nuclear inclusions can be usually found. Nucleoli are small to medium sized and eosinophilic. Mitoses can be present even in the deeper parts of the lesion. The proliferative index measured with Ki-67 antibody, is either absent or low. Pigment is present in melanophages that surround individual nests and bundles of melanocytes. Background presents usually focal mature lymphocytes and rarely plasma cells. Moderate cytological atypia and mitoses may be identified in some tumors; this and the lack of deep maturation could lead the pathologist to think about a melanoma. In summary the histological atypical features found in up to 40% of deep penetrating nevus include: asymmetry of the lesion, expansive melanocytic nests in the dermis, conspicuous eosinophilic nucleolus, absence of maturation, and inflammation. Deep penetrating nevi are may be present associated to common acquired melanocytic nevi, blue nevi and also Spitz nevus. The main differential diagnosis is with melanoma. The features that favor a diagnosis of deep penetrating nevus include: the clinical presentation on the head and neck area of young individuals (< 50yo), circumscription, wedge-shaped architecture, lack of clonal expansive growth, significant cytological atypia and nuclear pleomorphism, numerous mitoses (including abnormal forms). Immune-histochemical stains for S-100 protein and HMB-45 are positive deep penetrating nevus.

Deep penetrating nevus: key histopathological diagnostic features
- Well circumscribed, symmetric, reverse triangle architecture
- Epithelioid pale cells in nests surrounded by melanophages
- Fascicles of variably pigmented spindle cell admixed with melanophages
- Smaller nests in deep than those superficially
- Mitoses rare, always typical
- Focal slight maturation present

05E Mongolian spot and nevus of Ota and Ito

The Mongolian spot is a slate gray macular pigmentation present in the lumbosacral area in 90% of Asian population and in 20% Caucasians. Usually disappear with the age. Histologically the Mongolian spot consists of dendritic pigmented melanocytes scattered in reticu-
lar dermis or subcutaneous fat. The histology od Ito and Ota nevus is similar and it is characterized by a band-like disposition of melanophages mixed with dendritic melanocytes similar to that found in blue nevus in the upper dermis. Dermal fibrosis and melanophages may be present. Mongolian spot unlike the nevus of Ito and Ota is of lower cellular density, no fibrous reaction and few melanophages.

<table>
<thead>
<tr>
<th>Mongolian spot and nevus of Ota and Ito: key histopathological diagnostic features</th>
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<tr>
<td>• Dendritic pigmented melanocytes in dermis and subcutaneous fat</td>
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<tr>
<td>• Pigmented melanophages</td>
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<tr>
<td>• Fibrous response</td>
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**05F Neurocristic hamartoma**

Rare lesion that occur on the scalp but has been described elsewhere in the skin. It is a pigmented lesion that tends to aggregate along hair follicles characterized by spindle cell melanocytes admixed with dendritic blue nevus cells, schwann cells, melanophages within a dense collagenous stroma perifollicular and along the subcutaneous fascia.

**06 Congenital nevus**

Congenital nevi are defined as nevi present at birth and they are identified in 1-2% of newborn infants. The size of congenital nevi varies from few mm to many cm sometimes occupying large part of the body. Some congenital nevi extend to subcutaneous fat and they may involve muscle and bone. Histologically the congenital nevus present the same types of cells of acquired nevi. Neuroid differentiation is quite frequent. Some histological features are characteristic of congenital nevi: presence of nevus cells in the wall of veins and lymphatics or in endoneurium but also in sebaceous glands, eccrine coil and duct, the external root sheath and subcutaneous septa. Presence of single nevus cells in mid and deep reticular dermis and around the adnexa in characteristic. Not infrequently other benign mature neuroid proliferations may coexist with congenital nevi such as neurofibromatous and ganglioneuromatous patterns. About 10% of congenital nevi show an intraepidermal proliferation with dysplastic like features but true dysplasia is usually absent. Nodules of confluent melanocytes with
different dimensions may be observed in the superficial dermis, so-called proliferation centers or nodules (see forward). Halo phenomenon may be also present.

**Congenital nevus: key histopathological diagnostic features**  
- Different sizes from 1-5 cm to covering large skin areas (garment distribution)  
- Lentiginous junctional proliferation of melanocytes with focal slight atypia and nests  
- Dermal appendageal and subcutaneous involvement by B nevus cells  
- Multiple nests and single nevus cell diffusely arranged  
- Perivascular and perineural extension

**06A Benign proliferative nodules in congenital nevus**

The development of focal nodular proliferations within large congenital melanocytic nevi can simulate melanoma. Clinically they are described as black, brown, or pink papules or macules within the melanocytic congenital nevi, most often measuring less than 1.0 cm and only sometimes they become ulcerated. Proliferative nodules have increased cellularity, usually expansive growth pattern with pushing borders, and larger melanocytes than in the background melanocytic component. The cells are epithelioid or spindled with abundant cytoplasm. They have small nucleoli, frequent intranuclear pseudo-inclusions. Mitoses are rare, there is no necrosis. Sometimes nuclear pleomorphism can be observed, but it is not associated with increased mitotic activity. No pagetoid spread is seen. Some inflammatory cell infiltrate composed of lymphocytes can be present in the background. Both congenital melanocytic nevi and proliferative nodules express S-100 protein, HMB-45, and Melan-A. Interestingly, p16 and CD95 are positive in all proliferative nodules in one study; CD117 is intensely positive in almost all adjacent congenital melanocytic nevi. Proliferative index (Ki-67) is usually low in proliferative nodules. The nodule may be also present in Spitz nevus. The main *differential diagnosis* is with neonatal melanoma in nevus. The most important features to consider include: marked pleomorphism, high mitotic activity, abnormal mitoses and presence of necrosis.
A careful evaluation of these proliferations reveals that the nuclei show very delicate and regular chromatin and that nuclear cytoplasmic ratios are small. Another important pattern is the absence of pagetoid spread at the nevus shoulder. The dermal component show a propensity to mature and blend with the underlying dermal nevus cells. In differential diagnosis it is to considerer the melanomas in children are very rare and most frequently they arise in the deep component of a large congenital nevus.

Superficial atypical melanocytic proliferation in congenital nevi: key histopathological diagnostic features
- Lack of aggressive proliferation of the junctional and intraepithelial component
- Occasionally large abnormally located junctional nests
- Pagetoid spread limited to epidermis over the dermal component
- Cytological atypia and mitoses
- Irregular maturation
- Clinicopathological correlation and second opinion are important

07 Conjunctival melanocytic proliferations

Melanocytic lesions are not uncommon in the conjunctiva but they may present clinical and histopathologic problems. In a large series of 1643 conjunctival different lesions by Shiels et al. 53% were nevi and melanomas. Conjunctival melanocytic proliferations are classified as freckle, acquired or congenital nevi, stromal and episceral melanocytoses, primary acquired melanosis without and with atypia, primary acquired melanosis associated to nevi and conjunctival melanoma.

07A Freckles and ephelides of the conjunctiva are similar to those of the skin with increased pigment within basal conjunctival epithelium.

Conjunctival freckles or ephelides: key histopathological diagnostic features
- Hyperpigmentation of basal keratinocytes
- Normal epithelial architecture
- Melanocytes present in normal number or diminished

07B Conjunctival nevi

 Conjunctival nevi are present frequently in young individuals and situated near to the junction between sclera and cornea. Acquired conjunctival nevi arise on the bulbar conjunctiva and congenital conjunctival nevi may occur on both the upper and the lower eyelids. Histologically the conjunctival nevi may exhibit an intraepidermal junctional or a sub epithelial component or both. Frequently the nevomelanocytic component is densely pigmented and hyperchromatic. A variant of conjunctival nevus is associated with multiple apocrine cysts. Cyst like spaces with goblet cell are present in 70%
without atypia presents as flat non-cystic patches of the conjunctival pigmentation usually in patients over the age of 40 years.

**Primary conjunctival melanosis without atypia: key histopathological diagnostic features**
- Hyperpigmentation of basal keratinocytes
- Basilar melanocytic hyperplasia without atypia in lentig nous pattern.
- Rare nests may be present

**07D STROMAL AND EPISCLERAL MELANOCYTOSIS**
The stromal and episcleral melanocytoses are: **nevus of OTA** (oculo-dermal melanocytosis) with bipolar spindle dled or multipolar melanocytes that involve all or part of the uveal tract (iris, ciliary body and choroid) as well as the episclera; the **blue nevus** and the **cellular blue nevus** characterized by spindled-shaped melanocytes in conjunctival stroma with schwannian appearance and multiple combined nevi (acquired or congenital and blue nevi).

**References**

**01 Non-neoplastic hyperpigmentations**

**01A Ephelides**

**02 Acquired melanocytic nevi**

**02A Lentigo simplex**

**02B Junctional, compound and dermal nevi**


**07C PRIMARY CONJUNCTIVAL MELANOSIS**
Melanosis can be primary, secondary, congenital or acquired. The most common is the primary acquired melanosis (PAM) with or without atypia. Conjunctival melanosis may present three histologic patterns: hyperpigmentation of the basilar epithelial keratinocytes without melanocytic hyperplasia, or benign melanocytic hyperplasia with hyperpigmentation or atypical melanocytic hyperplasia with hyperpigmentation (PAM). The last may be considered a precancerous melanosis. PAM compound, 58% stromal (sub-epithelial) and 40% junctional nevi. Less than 1% of acquired conjunctival nevi progress to melanoma.

**Conjunctival nevi: key histopathological diagnostic features**
- Junctional nevi
  - Intact surface epithelium of normal thickness
  - Epithelial down-growths with or without cyst formation
  - Rare mitoses
  - Nest or linear arrangement of junctional melanocytes
- Compound nevi
  - Prominent epithelial down-growths with or without cyst formation
  - Superficial nests heavily pigmented
  - Maturation of dermal component
  - Stromal inflammatory infiltrate
- Sub epithelial-stromal nevi
  - No junctional component
  - Maturation
  - Cyst formation

**Fig. 52. Compound conjunctival nevus.**

**Fig. 53. Multiple apocrine cysts in compound conjunctival nevus.**

**07D STROMAL AND EPISCLERAL MELANOCYTOSIS**
The stromal and episcleral melanocytoses are: **nevus of OTA** (oculo-dermal melanocytosis) with bipolar spindled or multipolar melanocytes that involve all or part of the uveal tract (iris, ciliary body and choroid) as well as the episclera; the **blue nevus** and the **cellular blue nevus** characterized by spindled-shaped melanocytes in conjunctival stroma with schwannian appearance and multiple combined nevi (acquired or congenital and blue nevi).

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**01A Ephelides**


02C HALO NEVUS


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02E BALLOON CELL NEVUS


02F RECURRENT NEVUS


02G NEVUS WITH NEUROTIZATION


02H AGING (ANCIENT) NEVUS


02I COMBINED NEVUS


02K OTHER RARE VARIANTS OF NEVI


Nevus of Nanta (dermal nevus with ossification)


Elastic fibers in nevi

Nevus with trichostasis spinulosa

Nevus with pseudovascular lacunae

Nevus in combination with other neoplasms

Traumatized nevus

Nevus with myxoid change

Nevus with amyloid

Nevus with pagetoid cells

Nevus with pagetoid cells

Nevus with pseudovascular lacunae

Nevus with myxoid change

Nevus with amyloid

Nevus with pagetoid cells

Nevus with amyloid

 Lobulated intradermal nevus

Inverted type A nevus

Incidental nevus

Fat cells in intradermal nevus


Nevi in pregnancy and hormonal therapy

Nevus in childhood

Nevus in lymph node (nodal nevus)

03 Nevi of special sites

03A GENITAL NEVI


03B ACRAL NEVUS


04 Spindle and epithelioid cell nevi

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04B PIGMENTED SPINDLE CELL NEVUS (REED NEVUS)


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05A BLUE NEVUS


05B CELLULAR BLUE NEVUS


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06 Congenital nevi


06A Benign proliferative nodules in congenital nevi


06B Superficial atypical melanocytic proliferation in congenital nevi


Conjunctival melanocytic proliferations


Drug-induced gastrointestinal injury (DIGI). Updates, reflections and key points

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Key words
Drug-induced gastrointestinal injury • Identifiable drugs • Angiotensin receptor inhibitors • Immuno-modulators

Summary
The goals of this short narrative review are 1) to provide an update in recent developments in the field of drug-induced gastrointestinal injury (DIGI), and 2) to distill few key points to approach with confidence a difficult and vast area of gastrointestinal pathology. DIGI is a challenging diagnosis as it can produce almost any pattern of the injury of the gastrointestinal tract. The recognition of a pattern and the knowledge of which drugs can produce that pattern, are the first step of the diagnostic process; communication with the clinical team and a high level of suspicion are then paramount. The pathologist can be the leading clinicians of the care team in few situations in which she/he can recognize the drug at the microscope. Knowledge of the most relevant differential diagnoses of DIGI is essential to avoid significant pitfalls. Finally, several DIGIs due to recently developed immunomodulators used in oncology have shown relevance given their sometimes fatal outcome and the accumulating evidence of a common morphological appearance among them.

In alphabetical order:
AFB: acid fast bacilli stain; ARB: angiotensin receptor blocker; BAS: bile acid sequestrants; CMV: cytomegalovirus; CRF: chronic renal failure; DDC: dilated damaged crypt; DIGI: drug-induced gastrointestinal injury; GI: gastrointestinal tract; IBD: inflammatory Bowel Disease; I-C: inhibitor of CTLA-4 receptor; IELs: intraepithelial lymphocytes; GVHD: graft versus host disease; LC: lanthanum carbonate; NSAID’s: non-steroidal anti-inflammatory drugs; PI: PD1 receptor inhibitor; PI3K: phosphatidylinositol-3-kinase; SPS: sodium polystyrene Sulphonate (Kayexalate®)

Introduction
Drugs are known noxae with significant aggressive impact in the gastrointestinal tract (GI). Bates et al. 1 showed that the overall rate of adverse GI drug effects is 6.5 per 100 hospital admissions. Iatrogenic effects on the GI outside of conditions requiring hospital’s care remain unknown and are probably unknowable. To add, it is significant that doctors may not be aware of the existence, quality and/or severity of drug-induced GI injury (DIGI). Excellent reviews of DIGI are published almost yearly 2-4. This short narrative review intends not only to provide a comprehensive update on those previous works (to which we suggest strongly to refer to and upon which we build this update) but also, and above all, to crystallize in key points what we have learnt about DIGI along the years. The goal is to help the practicing pathologist to diagnose DIGI or, at least, to introduce DIGI in a meaningful differential diagnosis.

The basic knowledge
The interaction of drugs with intestine depends mainly on the drug’s physical-chemical characteristics, dosage, administration route, size of the pill, formulation (e.g.
sustained release) and on the recipient variables (e.g. other drugs that may interact, how the drug is ingested, the motility of the intestine, anatomic features, associated diseases). DIGI can be highly specific but more commonly it is not, thus requiring communications with clinical teams and temporal correlation with drug intake or drug stoppage to allow for diagnosis. Diverse controversial clinical situations can appear. On one hand, the intestine can respond to noxae with a very limited repertoire of histological responses/patterns (morphological “funneling”), i.e., various diseases or conditions may overlap in their histological manifestations. On the other, the opposite is also true, that is, one DIGI or one disease can “blossom” into multiple different histological patterns. When should the pathologist consider DIGI? Is DIGI frequent absence of diagnostic specificity a nihilistic sinkhole for the morphologist? In brief the answer is no. In general DIGI should be considered when an increase in eosinophils, in apoptotic activity, in intraepithelial lymphocytes (IELs) does appear, or when vacuolation of cytoplasm is noted in the mucosa cells.

We learned also that DIGI should be considered when: multiple portions of the GI are affected by various injury types (e.g. collagenous colitis and increased IELs in stomach), microscopic findings do not fit neatly into a known disease (e.g. apoptotic bodies that are markedly increased in what appears to be Inflammatory Bowel Disease, (IBD)), the clinical context is unusual [e.g. ischemic colitis in a young patient or ischemic gastritis], the patient is unresponsive to what appears to be an appropriate treatment (e.g. gluten free-diet in celiac disease). Nowadays it is fashionable to try to work up the differential diagnosis of gastrointestinal medical diseases using morphological patterns of injury; DIGI, however, escapes this approach as it is not a single disease and it can generate almost any type of injury pattern. The clinical background of the patient is essential not only because the clinician, hopefully, knows the drugs taken by the patient but also because knowing what conditions affect the patient may heighten the pathologist awareness of a DIGI. Certain conditions are treated with drugs that are well known to cause DIGI. A list of such diseases is in Table I.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Drugs used affecting GI</th>
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<tr>
<td>Chronic renal failure</td>
<td>Sodium polystyrene Sulphonate, Sevelamer, Lanthanum carbonate</td>
</tr>
<tr>
<td>Autoimmune disorders (e.g. lupus, myasthenia gravis, psoriasis)</td>
<td>Mycophenolate</td>
</tr>
<tr>
<td>Solid organ transplant</td>
<td>Taxanes, PDL-1 inhibitors, CTLA inhibitors, Adalylisib.</td>
</tr>
<tr>
<td>Cancer patients (CLL, FL, Melanoma, breast esophagus lung ovary cancer etc)</td>
<td>NSAIDs, Doxycycline, Clindamycin, Bisphosphonates</td>
</tr>
<tr>
<td>Polypharmacy, especially if in elderly or neurologically impaired patients</td>
<td>NSAIDs, Doxycycline, Clindamycin, Bisphosphonates</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Angiotensin Receptor Blockers (Olmesartan, Valsartan, Telmersartan)</td>
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A more common situation is that of a nonspecific GI ulcer. Drugs are considered the main causes of ulcerations of unclear origin routinely encountered in the GI. Ulcers due to drugs are in the overwhelming number of cases histologically nonspecific. Pill fragments are the most direct evidence of ulcer being a DIGI but this event is rare. Too numerous are the drugs capable of causing erosion/ulcer to be listed here, we provide instead clues for the differential diagnosis. Location of the erosion/ulcer can help. Esophageal “hang-up” areas (such as the aortic arch, the imprinting due to an enlarged left atrium, the gastroesophageal junction) lead to prolonged contact of the drug with subsequent chemical burn. Proximal esophagitis/ulcer is almost always pathognomonic of drug-induced esophagitis (lichen planus is the main differential diagnosis in these cases). The isolated proximal location effectively eliminates gastro-esophageal reflux disease as a cause. In addition to antrum and duodenal bulb, the right colon, and terminal ileum are locations for Non-Steroidal Anti-inflammatory Drug (NSAIDs) ulcers. In case of NSAIDs-induced diaphragms formation the ulcer would typically involve the tips of the diaphragms (Fig. 1).

Histology can provide some help: NSAIDs-induced ulcer is generally inflammation-poor, superficial, rarely involving the muscularis propria. Tetracyclines notorious esophago-gastro-toxic antibiotics include doxycycline: this antibiotic has gained possible morphological relevance. Doxycycline can cause erosions/ulcers in the entire upper GI but, uniquely so far, doxycycline can cause capillary vascular degeneration with a peculiar “necrotic” appearance and microthrombi (Fig. 2). In addition, as first described in the esophagus by Medlicott and Dupré, doxycycline causes a lymphocytic vasculitis with endothelitis and peculiar perivascular pallor due to edema and fibroblast proliferation (Fig. 3). Such pattern should promote an inquiry into doxycycline intake.

As previously stated drugs can induce all the pattern of

**KEY POINT #1:**
DIGI is most often non-specific histologically and can manifest with almost any pattern of injury. Clinical data, awareness and knowledge of DIGI pathology can help in the diagnosis.

**Tab. I. Clinical conditions that should alert the pathologist of drug-induced injury.**
injuries of the GI. Table III is an extensive list of offending drugs and related injury patterns in the various GI organs. Examples have been and will be highlighted in this short review but we defer to previous reviews on the topic for a more exhaustive discussion.

**When the pathologist is decisive: “GI mineralogy”**

Trained pathologists can astonish clinicians figuring out the patient intake of specific drugs relating them with several GI pathologies that can otherwise appear puzzling or unclear. Certain drugs can manifest as concretions, crystals or amorphous deposits in the GI (Tab. II). Unmistakable are the small and black 30 microns in diameter, round spheres loaded with yttrium-90 (Fig. 4) that are injected in the hepatic artery for internal radiation therapy directed at liver metastasis or hepatocellular carcinoma. An unfortunate diversion of these microspheres (for anatomical or procedural reason) displaces them in the upper GI organs or pancreas. Biopsies of the upper GI can easily detect them.

Pathologists will certainly not miss iron pills residues in the upper GI biopsies. Iron supplements (usually ferrous sulphate) at therapeutic doses can cause symptoms and DIGI in the upper GI in up to 16% of iron deficiency anemia patients. Iron deposits due to iron supplements are composed of layers of Perl’s stain-positive, fibrillary, yellow/brown to blue/black material on the mucosal surface and in the mucosa. Giant cells and, of course, hemosiderin-laden macrophages may be seen. Of note iron distribution in the stroma and in stromal histiocytes, or in glands, is not iron supplement-related, but follows previous ulcer or is due to hemochromatosis respectively. DIGI due to iron is almost exclusively in stomach and esophagus and is
itself erosive producing epithelial reparative changes. Erosions due to iron pills are instead very rare in the duodenum, where iron is seen in the reticuloendothelial cells in tips of villi. In the duodenum iron in lamina propria indicates therapeutic overload. Non-systemic/non-absorbable drugs exert their effects only in the lumen of the GI and are the logical target of microscopic recognition. Among this class of drugs are sequestering agents that act by forming complexes with a target molecule that can then be eliminated in the feces. The recognition of the crystals of these drugs is important as some of them can cause death or serious complications.

The best known offending agent among them is Sodium Polystyrene Sulphonate (SPS) (Kayexalate®), a cation-exchange resin used to treat hyperkalemia in chronic renal failure (CRF) patients. SPS, in the market for over 50 years, cannot be used chronically. SPS is administered suspended in sorbitol to prevent bezoar formation, via enema or per os or via nasogastric tube. Side effects occur regardless of the administration route.

Fig. 4. Unmistakable round, black yttrium-90 loaded particles lodged in gastric mucosa (approx. 30 micron in size).

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Crystals of SPS can be recognized in intestinal biopsies (Tab. II, Figs. 5, 6): they have a square to irregular outline, can vary from 10 to 200 microns or more in size, have a monotonous violet color on hematoxylin-eosin (H-E) stain, and contain regularly spaced “fish scales”-like lines. The best known SPS-side effect is acute ischemic ileitis and colitis in CRF patients. Initially sorbitol was thought to be responsible for all SPS-associated DIGI, however when SPS was used alone the risk of severe colonic ischemic injury was not eliminated. It remains advisable to use aqueous (not sorbitol) solutions of SPS. Crystals of SPS can be seen in the exudate or in the intestinal wall or even outside the organ (free or in inflammatory pseudotumors) if perforation has occurred. SPS injuries of the upper GI are either erosions or ulcers or other related diseases, i.e., emphysematous gastritis (see Fig. 6 for SPS associated with a rare case of esophageal pseudo-diverticulosis). SPS-related deaths have been seen almost exclusively after ileocolonic injury. New products are joining the market that can be used chronically (e.g. patiromer) and may have less risks for the patients.
Sevelamer carbonate (Rengela®, Renagel®) is a resin targeting phosphate ions used in the treatment of hyperphosphatemia of CRF or tumor lysis syndrome patients. Sevelamer causes chronic diarrhea and constipation. DIGI associated with sevelamer are reported in the upper GI (gastric pneumatosis, esophageal ulceration) and, especially, in the lower GI (colitis with crypt distortion and Paneth cell metaplasia, inflammatory polyps, recto-colonic ulcers, colonic stricture, colonic inflammatory mass formation and ischemia, colonic perforation of di-
verticulum)\textsuperscript{15-20}, although direct causation is not demonstrated. The features of the luminal crystals are shown in Table II and Figure 7. They are scaly in appearance with the crystal displaying a 2 toned-color appearance: pink and yellow (but also occasionally brown/red/purple). They stain magenta after Acid Fast Bacilli stain (AFB). SPS and sevelamer crystals identification will require a callback to the clinician. However, one must first exclude mimickers: the best known are other resins such as the bile acid sequestrants (BAS) (cholestyramine, colesevelam, cholestipol) crystals. BAS have been innocuous so far and their importance lies in their distinction from the ion-binder resins described above. Table II and Figures 5 to 7 provide the clues necessary for identification.

Lanthanum carbonate (LC) is a phosphate binder, devoid of aluminum and calcium, effective in the control of hyperphosphatemia in patients with CRF. No serious side effects have so far been linked to the use of LC. Makino et al.\textsuperscript{21} described LC deposition in gastric mucosa in CRF in 2015. LC deposition is frequent (seen in 14 of 19 CRF patients according to Goto\textsuperscript{22}) mainly in the stomach but also in duodenum\textsuperscript{23} and colon\textsuperscript{22}. LC has interesting characteristics: 1) is one of the few drugs causing an infiltrative cellular pattern of injury in the GI (another is clofazimine, a third tier anti-tuberculosis antibiotic); 2) it is radio-opaque and detectable using radiological imaging\textsuperscript{24} while displaying white/chalky granules or polyps at endoscopy with erosions and ulcers. LC is found in the lamina propria but also on the mucosal surface. The histology is that of a foreign body reaction characterized by an infiltrate of eosinophilic, large, often multinucleated, histiocytes containing colorless or brownish needle-like or branched, coiled or crescent-shaped inclusion bodies (Fig. 8). Formation of well-defined epithelioid granulomas (Fig. 8) is occasionally seen, highlighting again how a DIGI can mimic other conditions, in this case granulomatous gastritis. Regional lymph nodes have similar LC infiltrates\textsuperscript{24}.

The differential diagnosis of crystals/concretions/crystalline deposits in the GI has been enriched recently by additional compounds: crospovidone, microcrystalline cellulose and sodium phosphate oral tablets (OsmoPrep\textsuperscript{\textregistered}) creating a veritable “GI mineralogy” for the pathologist. Crospovidone (Fig. 9) and microcrystalline cellulose (Fig. 10) are not active principles: they are widely utilized drugs stabilizers and fillers that, when in oral medications, can be recognized in the GI, especially in the small intestine (in 9% of patients according to Shaddy et al.\textsuperscript{25}).

Crospovidone ranges from 0.4 to 1.5 mm in size, has a coral shape, generally with pink cores and purple coats in each segment on H-E (Fig. 9). It is not birefringent under polarized light and is dark orange after von Kossa stain. Microcrystalline cellulose is instead brightly birefringent under polarized light, and is transparent in H-E-stained slides (Fig. 10). These two compounds are well known to pulmonary pathologists as their presence in...
the lung indicates aspiration or intravenous drug abuse. The appearance in the lungs and GI is however different: while in the lung crospovidone is solid blue or black on H&E-stained sections, in the GI has a two-toned color. Sodium phosphate tablet OsmoPrep (USA: OsmoPrep®, France: Colokit®) is used for colonoscopy preparation, occasionally employed in patients that do not wish to ingest the large volumes of liquids otherwise required for the procedure. The drug in its solution preparation (Phosphosoda) is well known to cause colonic aphthous ulcers and focal active colitis. The tablets instead cause deposits in the stomach where they mimic calcinosis and iron-pill injury. The injury is of the gastropathy/erosive type. The deposition occurs in the gastric superficial lamina propria where it appears similar to crushed pill fragments, purple to black, less often translucent, less than 100 microns in size (Fig. 11). OsmoPrep is von Kossa stain positive (von Kossa stains the phosphate moiety) but alizarine red negative. This staining pattern distinguishes OsmoPrep from gastric calcinosis in which calcium is reactive for both stains.
Finally, the pathologist should be aware of the possibility of multiple compounds deposits and crystals being present in the same patient. The clinical background in which the drugs are used may also heighten the need to look for and/or exclude associated conditions (e.g. amyloid in case of CRF with GI resins crystals).

**KEY POINT #2:**
The pathologist can be decisive and recognize drugs in H-E stained slides. Identification is clinically useful.

**Some drug effects are traps we must know about**

Olmesartan medoxomil, a widely-prescribed angiotensin II receptor blocker antihypertensive drug, is used by millions of patients, a minuscule minority of them develops a significant DIGI after months to years of exposure. Olmesartan can cause sprue-like enteropathy especially in elderly patients. Partial or total villous blunting, increase in IELs and collagenous enteritis are reported frequently in this DIGI. The stomach and colon have similar findings consisting of lymphocytic and collagenous gastritis and colitis in isolation or associated with enteropathy (Fig. 12A). A detailed exam of the cases in the literature shows however that the occasional patient may sometime present without duodenal IELs increase (Fig. 12A) in olmesartan-induced villous atrophy, a significant difference with celiac disease. The recognition of this DIGI is perhaps the most important of all. The patients are often hospitalized with uncontrollable diarrhea and electrolytes disorders, typically with a diagnosis of sero-negative celiac disease; “suspicious for lymphoma” is another preliminary diagnosis that will also be given due to the refractoriness to all therapies and the severity of symptoms. The suspension of olmesartan will prevent malnutrition and expensive testing, and is spectacularly effective in relieving the symptoms in few days and in restituting the morphology to normal, or almost normal, in few months. Symptoms do recur after reintroduction of olmesartan in the few cases in which this was attempted. Of note other members of the Angiotensin Receptor Blocker (ARB) family are capable of causing severe enteropathy and enterocolitis (e.g. Valsartan and Telmersartan) and Losartan (Dr De Marco personal communication). The dramatic enteropathy certainly dominates our clinical interaction with ARBs DIGI, however it is likely that ARBs have milder histological presentations (e.g. increase in chronic inflammation, isolated duodenal or colonic IELs increase).

Taxanes (Paclitaxel, Docetaxel, Cabazitaxel) used in cancer patients (breast, ovary, digestive and prostate cancers) disrupt mitotic tubules preventing tubule depolymerization (mitotic arrest-drugs) and have a direct antitumoral apoptotic effect (Docetaxel in particular). Taxanes can mimic dysplasia anywhere in the GI and to perfection in mucosa of Barrett’s esophagus. Atypical
nuclei with numerous mitoses (some appearing as ring mitoses, some others with a central bar) and increased apoptosis are the results of taxanes intake (Fig. 12B). To help in the differential diagnosis between true dysplasia and taxanes it has been reported how taxanes affects only the proliferative areas of the glands and that hyperchromasia and nuclear pleomorphism are absent in taxanes effect 31. The differential diagnosis remains highly difficult in Barrett’s esophagus or other conditions prone to dysplasia and exposed to taxanes: only awareness of this DIGI and clinical correlation will help prevent this pitfall.

Mycophenolate is used in solid organ graft maintenance therapy and in autoimmune disorders (e.g. lupus, psoriasis, myasthenia gravis). Mycophenolate colitis is well described 2-4: the main features are (Fig. 12 C) architectural disarray of the mucosa with crypts drop out and “exhausted” apoptotic crypts (that is: thinned stretched eosinophilic epithelium around a dilated/damaged-appearing crypt (DDC) with eosinophilic luminal debris and increased apoptotic bodies) 4,32,33. Occasionally mycophenolate colitis is ischemic in appearance, in individual cases the colitis is worse in the proximal colon. Of note the colitis can have skip areas further mimicking Crohn’s disease to the unaware. In the small intestine villous atrophy and inflammation due to mycophenolate can mimic celiac disease in the individual case. The DDC pattern of mycophenolate is reminiscent of, and needs to be distinguished from, cytomegalovirus infection and especially GVHD. These conditions must be excluded due to vastly different therapies. Star et al. 34 summarized features to help distinguish the two as follows: mycophenolate colitis features much more prominent lamina propria inflammation with eosinophils than
GVHD, while GVHD displays endocrine nests and apoptotic microabcesses more commonly than mycophenolate.

**KEY POINT #3:**
The most relevant pitfalls due to DIGI are those related to olmesartan and other ARBs (vs celiac disease and/or collagenous enteropathy), mycophenolate (vs. GVHD and CMV) and taxanes (vs. dysplasia).

**Oncology is a fertile ground for DIGI**

New oncologic therapies are not exempt of side-effects in GI. DIGI provoked by the recently developed immune checkpoint inhibitors and Phosphatidylinositol-3-Kinase (PI3K) inhibitors will be analyzed here.

**Immune checkpoint inhibitors.** Under this category are CTLA-4 (cytotoxic T-lymphocyte associated protein-4) inhibitors (ipilimumab and tremelimumab) and Programmed Death-1 receptor inhibitors (PDI) (such as pembrolizumab, nivolumab and atezolizumab that targets the PD-1 ligand). These two classes of inhibitors are sometimes used together in modern oncology therapy. The severity of the GI side effects is higher after CTLA-4 inhibitors 35-41.

CTLA-4 is a receptor whose activation inhibits cytotoxic T-cells. Inhibitors of CTLA-4 (I-C) remove this inhibition and prolong cytotoxic T-cells activation and action on tumor cells. I-C are approved for the treatment of advanced melanoma, they are under investigation in the treatment of non-small cell carcinoma of the lung, as well as prostate, renal and ovarian carcinoma, and in mesothelioma. The mechanism of action of I-C leads to loss of systemic tolerance and a host of immune-related disorders including DIGI. Between 20 and 50% of patients suffer GI side effects, the most common being watery diarrhea appearing within 6 weeks into treatment sometimes as early as few days from the beginning of the therapy 35.

Life threatening colitis is seen in 16% of patients36. Less than 1 percent of patients suffer serious intestinal side effect or death 36. The endoscopic appearance of diffuse colitis was seen in 21% of patients in a study by Beck et al. 37. Ipilimumab offends also stomach and small intestine, sometimes in isolation. Endoscopy shows granularity, exudate, and erosion/ulcers but can be entirely normal even though colitis is present: biopsies must always be obtained. The histopathological findings 38-39 are similar throughout the intestine. The histology demonstrates villous blunting, diffuse chronic inflammation of mucosa, and increase in apoptosis, rare neutrophilic cryptitis, and intraepithelial lymphocytosis. They resemble autoimmune enterocolitis, an otherwise utterly rare disease in adults. The diagnosis of AIE in an adult should be made after DIGI has been excluded. The process may occasionally suggest IBD on a superficial observation, however in the colon there are no classic IBD findings such as basal plasmacytosis and crypts distortion. As a reminder, classic IBD would not have severe apoptosis or marked increase in IELs (Fig. 13). Rare is lymphocytic colitis and even rarer is ipilimumab associated lymphocytic plexitis of the colon. An exceptional case from Mayo Clinic showed the activation of a latent celiac disease into overt disease after ipilimumab 40.

PD-1 receptor activation prevents cytotoxic T-cell activation by self-antigens. Tumors use this mechanism to defuse the antitumoral immunological response. PD-1 inhibitors (PI) unbridge the T-cell response to malignancies. PI are employed in melanoma, urothelial carcinoma, non-small cell carcinoma of the lung, renal carcinoma and mismatch repair deficient colorectal carcinoma. It is not surprising that the enhanced T-cell response is accompanied by a series of side effects that appear to be immune-related. PIs cause mild watery diarrhea in 20-30% of patients, approximately 6 weeks into treatment 41 (severe in less than 10% of cases), and other GI symptoms (nausea vomiting, abdominal pain occult blood in stool. The endoscopy shows erythema friability and aphthous ulcers. The histology of PI injury has been recently described in 19 patients 42. Any portion of the GI was found affected in at least one patient. The upper GI showed increased mononuclear cells in the lamina propria of stomach and duodenum, villous blunting, increased eosinophils, neutrophilic villitis, neutrophils in foveolar epithelium. On the other hand, increased apoptosis was less common. Similar findings were seen in the terminal ileum. Distortion of mucosal architecture was not a feature of PI DIGI. The colon had similar findings with, however, more common apoptotic bodies and, interestingly, ischemic colitis. Increased intraepithelial lymphocytes were seen in both upper and lower GI. Collagenous colitis was seen in one case. An unusual finding was the development of well-formed granulomas associated with crypt ruptures.

**Phosphatidylinositol-3-kinase (PI3K) inhibitors.** PI3K are a family of kinases that regulates several cancer cells functions. Idealisib (Zydelig®) is a selective inhibitor of the PI3K delta isoenzyme that promotes apoptosis in cells of hematopoietic malignancies. Idealisib is used in therapy of relapsed or refractory chronic lymphocytic leukemia, small lymphocytic lymphoma and follicular lymphoma. Idealisib therapy can causes immune-mediated toxicities affecting liver and colon 43, side effects that may require cessation of the therapy. Diarrhea is usually watery and appears in 20-45% of patients 44. Early diarrhea (in the first two months) is responsive to symptomatic therapy without need of stopping the drug. Late diarrhea is considered a symptom of Idealisib-induced autoimmune enterocolitis and generally requires discontinuation of the drug 44. Endoscopy of the colon often reveals nothing of relevance or, in a minority of cases, pseudo-membranes, aphthous ulcers and erythema. Histological changes in the small bowel include increase in mononuclear cells infiltrate in lamina propria, increased apoptosis, villous atrophy, and increased intraepithelial lymphocytes, less commonly reduced gob-
let cells, and acute inflammation. Histological changes in the colon go under the umbrella of several injury patterns: GVHD pattern, IBD pattern, acute colitis pattern, and intraepithelial lymphocytosis pattern. Among the constitutive abnormalities, apoptosis and neutrophilic inflammation are uniformly found in every case. Interestingly dilated/damaged exploded/apoptotic crypt with luminal debris with few neutrophils is often part of the inflammatory process. These changes mimic other immunomodulators and mycophenolate effects and conditions such as Common Variable Immunodeficiency in the GI, autoimmune enterocolitis, GVHD and CMV colitis.

It appears that modulation of immunological mechanisms causes an overall repetitive pattern of injury in the various situations and drugs listed above. This allows a generalization into our final key point:

**KEY POINT #4:**
Apoptosis, increase inIELs, mild neutrophilic cryptitis and chronic inflammatory infiltrate are the common traits of colitis due to immune checkpoint inhibitors, PI-3-Kinase inhibitors and mycophenolate. Villous shortening and IELs increase are the common traits of these drugs enteropathy. The overall pattern of immunomodulators DIGI has significant overlap with autoimmune enterocolitis, celiac disease, Common Variable Immunodeficiency, even IBD. In an oncology patient, these drugs effects should be excluded before alternative diagnoses are offered. CMV and GVHD, in the appropriate clinical setting, must also carefully be considered and investigated.

**Final words**

This review intended to provide direct and useful messages and reflections in a field that by its variegated nature is otherwise of difficult synthesis. Key points proposed integrate clinical and pathological data making the pathologist a crucial element in DIGI recognition and appropriate therapy. The information and ideas conveyed have been collected from personal experience and literature review, hopefully they enlighten the reader with regards to DIGI and provide means to avoid the main DIGI traps and pitfalls. The future will hold undoubtedly new challenges from DIGI for the GI pathologist. Old drugs may be replaced by new and safer ones (see SPS being replaced by less dangerous drugs for example), but unwanted effects of new drugs will need to be ascertained. We have not mentioned several drug-related effects (e.g. Colchicine vs taxanes, Dasatinib, TNF antagonists and many more): we wanted few concepts to be in the forefront not an encyclopedia of DIGI; having said that we also think that a repository of the pathology of DIGI (similar to that of Drug Induced Liver Injury) would certainly benefit the pathology community facing this ever increasing and complex challenge.

**Appendix**

Drugs mentioned in the paper and relative brand names: The commercial names listed are by no means intended to be complete. E.g. multiple agents may be present together in association with the offending drug considered here for DIGI (for example with olmesartan). The list is
only meant to be helpful to recognize some of the most commonly used name brands associated with the drugs mentioned in the paper. NSAIDs (a very long list of aspirin and COX inhibitors could be entered here, please refer to textbooks or websites), doxycycline (Vibramycin® and many other brand names); Yttrium-90 loaded particles (Syrtex SYR-spheres®); Iron supplements (several formulations and brand names), Sodium polystyrene sulphate (Kayexalate®); Parietor (Veltassa®); Sevelamer (Rengela®, Rengan®); Cholestyramine (Questran®, Prevalite®); Colesevelam (Welchol®); Cholestipol (Colestid®); Lanthanum carbonate (Fosrenol®); Sodium phosphate tablets (OsmoPrep®, Olmesartan (Benicar®, Azor®) etc); Valcophenolate (Mycophenolate mofetil: Cellcept® and Mycophenolate sodium: Myfortic®); Ipilimumab (Yervoy®); Patiromer (Veltassa®); Sevelamer (Rengela®, Rengan®); Sodium polystyrene sulphate (Kayexalate®) in sorbitol: an underrecognized condition. Am J Surg Pathol 2013;3:521-3.

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Discovering intratumor heterogeneity: the next frontier for pathologists

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Key words
Intratumor heterogeneity • Pathology • Diagnosis • Oncology • Targeted therapy

Summary
Discovering intratumor heterogeneity is a crucial issue in modern oncologic medicine. Highly sophisticated technology such as high-throughput DNA sequencing has demonstrated the real dimension of the problem. The overwhelming majority of malignant tumors show high levels of intratumor heterogeneity when thoroughly studied. Intratumor heterogeneity develops both in temporal and spatial domains and its distribution is not deterministic making each case truly unique and unrepeatable. Pathologists are main actors in intratumor heterogeneity detection since they are the medical specialists who sample the tumors. Recent evidences have shown that currently applied sampling protocols are insufficient for a reliable intratumor heterogeneity detection. Pathologists must adapt classic sampling to the new times thus continuing being key pieces in the multidisciplinary approach to neoplasia that modern medicine demands.

Introduction
Intratumor heterogeneity (ITH), namely, the fact that a given neoplasm is qualitatively different along its different regions, is an intrinsic characteristic of human malignancies with high impact in modern medicine. ITH is responsible for most of the current therapeutic failures and constitutes the main obstacle to improve survival in patients with cancer. Unveiling ITH is not an easy task in clinical practice. It is a complex and multifactorial problem involving basic researchers and clinical specialists. Pathologists are main actors on the clinical side of the care of cancer patients, since they are responsible for the initiation of the solution to the problem. Pathologists handle surgical specimens and decide which specific parts of the tumor are going to be analyzed, and this is a crucial decision for the patient. Any non-detected molecular alteration in an insufficient or incorrect tumor sampling will be lost forever when the remaining of the surgical specimen is eventually discarded.

This mini-review intends to call the attention of pathologists to the imperative need of identifying ITH providing scientific evidence helping pathologists to abandon old habits and move forward to design more informative sampling.

Scientific approach
Pathologists look at the tumors by the naked eye and under the microscope. Maybe because of that (and usually under a high clinical pressure), pathologists have a biased perception of what is actually a neoplasm. Diagnostic routine tends to lead the pathologist to consider tumors as mere static two-dimensional groups of cells with distinctive morphologies and architectural arrangements. There is a permanently updated body of knowledge translating morphologic data to pathological diagnoses which, in turn, will conditionate the implementation of specific treatments. However, a neoplasm is something more complex. A neoplasm is a community of billions of cells dynamically interacting with each other. The collective behav-

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ior of these modified cells has deep roots into theoretical physics and follows nonlinear oscillatory far from the equilibrium self-regulated models condensed in power laws 6 7. Following these models, a neoplasia, a colony of bacteria, and a community of bees, for instance, display similar behavioral patterns. These patterns are condensed in the concept of *swarming* 8. Swarming refers to a collective unison behavior of large collectivities of insects, fishes, birds (and cells) without any centralized coordination. Swarming behavior is not exclusive of biological phenomena; events as diverse as earthquakes, robotics, traffic in big cities and stock exchange fluctuations follow similar behaviors 7. There are mathematical models demonstrating that the underlying rules for tumor metastatic settlements and the establishment of new ant colonies are the same. In fact, the laws of physics that govern neoplasia are the same that regulate not only all biology, but also the entire universe. Actually, physicists call *dark matter* the set of ions and proteins contained in the cell attributing them central roles in the development of many of their activities 9.

The possibilities for developing mutations in such a huge community of cells are practically infinite but the rules that govern this phenomenon are totally unknown so far. The classical approach establishes that the evolution of malignant cells in a neoplasm follows a Darwinian pattern 10. However, non-Darwinian models of evolution have been detected in highly heterogeneous neoplasms 11, thus indicating that the *decisions* adopted by malignant cells to accomplish ITH might be much more complex than believed 12, since they are not genetically determined 13.

The development of ITH is unclear and the collected data are contradictory so far. ITH is a stochastic spatial and temporal event 14 14. Spatial ITH leads to tumor regionalization resulting in different tumor areas with different mutational statuses. This leads to the development of branching patterns specific of each tumor 14. Temporal ITH, however, is made of not time-dependent events that seem to develop at very early stages of carcinogenesis 15. These findings contradict the widespread belief that a tumor is more homogeneous the greater and more evolved it is and places the focus of interest in small tumors, many of them incidentally discovered in daily practice.

**Clinical approach**

No doubt, ITH is the next frontier of modern oncology. An enormous number of scientific papers have been trying to decipher it in the recent years, but the problem is far from being fully elucidated. When examining current approaches, it seems that modern medicine has not noticed the importance of the discovery of ITH for the patient. For instance, ITH is right now the main obstacle for the development of targeted therapies in many types of cancer. The success of these therapies depends on the non-responder component of the tumor, that is some cases has not been identified, sampled, or characterized enough. This situation leads to oncologists to demand urgent solutions 8. To overcome these limitations, various authors have recently developed algorithms to assess ITH when there is very little material to analyze 16-19.

The problem is generalized and goes beyond pathologists. On one hand, it is a technological problem. Massive sequencing devices are still very expensive and data mining requires specialization in bioinformatics, two aspects that are far from being adopted by many public hospitals. On the other hand, the problem is the representativeness of the samples. Are endoscopic, transthoracic or transrectal biopsies representative enough so as to give something else than a morphological diagnosis 20 21? Can a targeted therapy be initiated with the molecular information obtained from small tissue fragments representing less than 1% of the tumor? 22.

To overcome this hurdle, and to make easier obtaining a sample by non-invasive methods, the liquid biopsy is being postulated as a safe alternative. Liquid biopsy can detect free DNA and circulating neoplastic cells in peripheral blood and its usefulness is right now being tested in colorectal 23 and breast 24 cancers. However, its real applicability is still to be demonstrated.

Recent literature shows many examples of confusing results obtained in molecular analyses of the same tumor types and, even more important, deep disagreements in the appropriateness of providing expensive therapies. In this specific scenario, pathologists should first wonder how many of these apparent inconsistencies might be simply due to incomplete/inadequate tumor samplings. Even after the problem of the sample representativeness is considered (and understood as not yet solved), the clinician must then face another issue: Individual intrinsic tumor resistance to treatments. The presence of cell clones resistant to targeted therapies can be either *de novo* or therapy induced 25-29. Another strategy in development is the so called adaptive therapy, in which the objective is to maintain stable the mass of cells sensitive to therapy via variations in the treatment 30. The goal here is to achieve, if not cure, at least the non-progression of the tumor.

**Pathological approach**

Most targeted therapies are introduced once the pathologist report is available. When a neoplasm cannot be studied *in toto* due to its large size, pathologists make a selection of the samples to be analyzed based on accepted and recommended protocols. However, these protocols were designed in a dogmatic fashion when ITH was not an issue and therefore need urgent updating.

Pathologists around the world face the same questions when facing a large tumor: where to sample and when to stop sampling? Deficiencies in molecular ITH detection, together with the lack of an appropriate therapeutic response, are weakening the position of pathologists in the diagnostic process. The adoption of appropriate guide-
lines to identify ITH in pathology laboratories seems mandatory and urgent. The main efforts of pathologists are directed towards microscopic and molecular diagnoses while tumor sampling is perceived as a task of somewhat lesser importance. The question seems simple, but it is still not answered: What is a correct tumor sampling? Fukuoka et al. 31 have developed a new method called spiral array that improves ITH detection in formalin-fixed paraffin-embedded samples of lung 32 and prostate 33 cancers. Spiral array allows tissue optimization in biopsies with scarce material in their retrospective analysis. However, spiral array does not resolve tumor sampling in a prospective manner. Multi-site tumor sampling (MSTS) 34-36 has demonstrated that 60-80 small samples obtained from a tumor placing them in the same number of cassettes that would be used by routine protocols through diminishing their size 34. In this manner, a 10 cm in diameter tumor would make use of the same 10 cassettes (1 cassette per centimeter of tumor) with six to eight small fragments on each cassette. Common sense, an in silico analysis 34, and a preliminary clinical validation 36, demonstrate that 60-80 small samples (in 10 cassettes) obtained from many tumor areas across the tumor detect much more ITH than 10 large samples (in 10 cassettes). Should molecular analysis confirm this data MSTS could be an affordable solution balancing sustainability and efficiency.

Conclusions

Heterogeneity is inherent to life, and tumors are no exception. Pathologists have the main responsibility for the detection of ITH since they are the specialists handling surgical specimens. Current sampling protocols are inefficient at detecting ITH and must be updated. The success of many expensive targeted therapies depends on the ITH identification. For these reason, discovering intratumor heterogeneity is the next frontier for pathologists.

References


Case report

Lane’s type pseudosarcoma of glans penis

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Key words

Pseudosarcoma • Immunohistochemistry

Summary

We herein present a rare case of polypoid “pseudosarcomatous lesion” of the glans penis, associated with in situ or mini-invasive squamous carcinoma. These lesions, described by Lane, in the upper aerodigestive tract, can rarely occur elsewhere. Immunohistochemistry is crucial for a correct diagnostic interpretation, confirming that the atypical cells are components (fibroblasts, myofibroblasts, endothelial) of granulation tissue.

Introduction

Aim of this report is to present a peculiar and controversial lesion in a location so far never described, and on the basis of our data and those of the literature make some interpretative considerations.

Case report

A seventy-nine-year-old man, has had for about 3 months an ulcerated, pedunculated polypoid mass on the ventral aspect of the glans penis in correspondence of the frenulum, the size of 2.2 cm x 1.5 x 1. Resistant to local therapies. The lesion is excised in toto.

Materials and methods

At macroscopic examination the surgical specimen was oval shaped, measured 2.2 x 1.5 centimetres x 1. The cut surface of it appeared pink, moist, granular, the outer surface smooth, covered by a layer of fibrin- necrotic material.

The entire sample, formalin-fixed and paraffin-embedded, was stained with hematoxylin-eosin and tested immunohistochemically with the following antibodies: vimentin (monoclonal 1:50 DAKO), CK AE1-AE3 (monoclonal 1:50 DAKO), EMA (monoclonal 1:50 DAKO), alpha-smooth muscle actin (monoclonal 1:50 DAKO), calponin (monoclonal 1:50 DAKO), CD68 (ready to use DAKO), S100 (polyclonal 1:400 DAKO), Ki67 (monoclonal 1:75 DAKO), CD34 (monoclonal 1:20 DAKO), CD31 (monoclonal 1:20 DAKO), P63 (monoclonal 1:50 Dako).

Histology

The surface of the neoformation appears widely ulcerated. The residual squamous epithelium is strongly atypical with the characters of in situ carcinoma (Figg. 1, 2). The bulk of is formed by a connective tissue rich in vessels, some thrombosed with promi-

Tab. I.

<table>
<thead>
<tr>
<th>CK Ae1-ae3</th>
<th>Ema</th>
<th>Vim</th>
<th>Smact</th>
<th>Calp</th>
<th>Cd68</th>
<th>Cd34</th>
<th>Cd31</th>
<th>S100</th>
<th>Ki67</th>
<th>P63</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surf + Fig.7</td>
<td>Surf +</td>
<td>SS +</td>
<td>SS + Fig. 8-9</td>
<td>SS + Fig. 10</td>
<td>SS+ Fig. 11</td>
<td>SS + Fig. 12</td>
<td>SS+</td>
<td>-</td>
<td>Surf+ Fig. 13</td>
<td>Surf+ Fig. 14</td>
</tr>
</tbody>
</table>

Vim=Vimentin, Smact= Smooth muscle actin, Calp= Calponin, Surf= Surface, SS = Sarcomatoid Stroma

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**Figs. 1, 2.** Strongly atypical superficial squamous epithelium, with the characters of in situ carcinoma. **Figs. 3, 4.** Stromal atypical elements: spindled, ganglion-like, multinucleated Touton-type. **Figs. 5, 6.** Thrombosed vessels with prominent endothelium.
nent endothelium (Figg. 5, 6, 13, 14) and, by a brisk proliferation of pleomorphic, atypical elements: spindled, ganglion-like, multinucleated Touton-type (Figg. 3, 4). The mitotic activity was sporadic, sometimes atypical. The immunohistochemical analysis shows immunoreactivity for epithelial markers (cytokeratins; EMA) restricted to the overlying squamous epithelium, while the atypical, pleomorphic stromal elements showed diffuse positivity for vimentin, alpha-smooth muscle actin, calponin CD68 CD34, and CD31 P63 and Kit67, staining was limited to the squamous epithelium.

Discussion

In 1957 N. Lane reported a series of 10 cases of polypoid lesions (Sarcoma-like) associated with carcinoma of the mouth, fauces and larynx. “These had in common the presence of an intramucosal or invasive squamous carcinoma, which was often incohesive and easily overlooked, associated with an anaplastic stroma of sarcomatous appearance, which was often bulky and preponderant… In the author’s cases the “sarcomatous” stroma is regarded as a secondary reactive phenomenon and perhaps is interpreted as probably non-neoplastic” 1.

Since that publication, Pubmed lists 62 reports concerning this lesion. Of these 34 had esophageal location, 28 pharyngo-laryngeal, 2 lingual 2, 11, 2 anal 12, 13, 1 oral 5 and 1 Cervical uterine 9.

To date no reports of this type of lesion on the external male genitalia are present in the literature.

The distinctive features of these lesions are: the polypoid appearance the surface epithelium of non-keratinising squamous type, the ulceration, the coexistence of a squamous cell carcinoma, usually in situ or minimally invasive, the predominant localization at the level of the upper aerodigestive tract.

It should be emphasized, however, that the other locations, although rarely, may be mucosal membranes with a structure similar to those of the upper aerodigestive tract.

Over the years there has been an active debate about the meaning of the “sarcomatous” component.

Some authors, following the opinion of Lane, considered it as a reactive process, while others as neoplastic in nature (sarcomatoid carcinoma). With regard to the lesions of the pharyngo-laryngeal district, a deeper analysis of the abstracts points out that about 50% of the authors are oriented towards the diagnosis of sarcomatoid carcinoma or carcinosarcoma, while the remaining 50% are inclined to believe that the “sarcomatous” component is of reactive nature of 1 1 1-5 7 9 11 22.

As far as the esophageal lesions are concerned, 60% of the authors are oriented towards a diagnosis of sarcomatoid carcinoma or carcinosarcoma, 32% tend to consider it as a reactive lesion 8 15-20 23.

Actually a careful evaluation of the morphological data allows us to differentiate the lesion described by Lane from the true carcinosarcomas and sarcomatoid carcinomas. The former has always polypoid morphology, it occurs in areas covered by non keratinising stratified squamous epithelium, and it is not associated with a non- or mini-infiltrating epithelial, proliferative lesion. The sarcomatoid carcinoma is usually widely infiltrating. These concepts were clearly expressed, several years ago, in a previous publication by one of the authors of this report 3.

The debate lasted until the advent of the immunohistochemical survey making it possible to separate the true carcinosarcomas from sarcomatoid carcinomas, from reactive lesions, although, in truth, in the most recent literature the problems do not seem completely solved. This happens, in our opinion, when those morphological and histogenetic differences, between different pathological entities, which we have specified above, are not clearly borne in mind.

An exhaustive description and immunohistochemical evaluation of the sarcomatoid carcinoma of the penis is found in a report 21 on the study of 15 cases. The macroscopic description is that of a vegetating (fungantig) tumor, often warty and largely infiltrating. In this tumor the sarcomatoid elements coexpress epithelial (cytokeratin, EMA) and mesenchymal (vimentin) markers, in the absence of myogenic and angiogenic markers. In our case the profile is quite different as the so-called “pseudosarcomatous” component is entirely negative to the epithelial markers, while it is polimmunophenotypic in nature, being composed of fibroblasts, myofibroblasts, endothelial cells. This cellular composition perfectly replicates that of a granulation tissue.

In the final analysis the immunohistochemistry fully confirms what was suggested by Lane in his seminal report.

Conclusions

We herein report a unique case of “pseudosarcomatous” lesion on the external male genitalia.

In our opinion we think that the process arises as a result of a pathological process involving squamous epithelium, including (dysplasia/ca in situ) or minimally invasive carcinoma. This condition is responsible of epithelial erosion/ulceration which induces, in the subepithelial stroma, the formation of a granulation tissue containing atypical fibroblasts, myofibroblasts and endothelial cells.

This confirms Lane’s opinion that this is a reactive process that, could be better defined as an atypical granulation tissue in the variegated world of pseudosarcomas, worthy of the eponymous of Lane’s Tumor.
Fig. 7. Cytokeratin. Figs. 8, 9. SMActin. Fig. 10. Calponin. Fig. 11. CD68. Fig. 12. CD34.
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**Case report**

**Follicular dendritic cell sarcoma of the head and neck. Literature review and report of the tonsil occurrence in a Ugandan patient**

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**Key words**

Palatine tonsil • Follicular dendritic cell sarcoma • Extranodal haematological malignancy • Immunohistochemistry

**Summary**

We report a case of follicular dendritic cell sarcoma (FDCS) in a 60-year-old Ugandan female who presented with a 6-year history of a progressive left sided tonsillar mass. General systemic examination was unrevealing and the patient underwent left tonsillectomy. She was subsequently lost to follow-up. Grossly, the mass measured 6 cm in diameter and had a mottled appearance due to tissue microhaemorrhages. Markers specific for follicular dendritic cell differentiation (CD21, CD35 and CD23), p53 and EGFR were expressed on immunohistochemical analysis.

Review of all of the 49 published reports of tonsil FDCS showed that this entity tended to occur at younger age (mean: 44.5 yrs) in women than in men (mean: 49.4 yrs). Tumour size ranged from 0.8 to 5 cm in maximum dimension (mean 2.9 cm). Only 12.2% of the patients presented with metastatic disease at initial diagnosis, all localised in the cervical lymph nodes. Local or distant recurrences occurred after a mean period of 72.5 months.

In conclusion, although the pertinent literature suggests that FDCS should be considered at least of intermediate grade, our review indicates that FDCS of the tonsil region behaves as a low-grade sarcoma.

**Introduction**

Recently discovered follicular dendritic cell (FDC) precursors in the lymph node are sessile cells originating from the vascular wall stroma and expressing platelet-derived growth factor receptor beta. Follicular dendritic cells are nonphagocytic, antigen-presenting immune accessory cells, located in the germinal centres of primary and secondary follicles, which may undergo sarcomatous changes. Follicular dendritic cell sarcoma (FDCS) accounts for only 0.4% of soft tissue sarcomas, with only 400 cases reported in the English literature, but has significant recurrent and metastatic potential and is considered by most authors an intermediate grade malignancy. The tumour usually occurs in the lymph nodes of the neck, mediastinum, and axilla. In approximately 30% of cases, the tumour develops in extranodal sites, preferentially in the head and neck area. In 1996, Nayler et al. reported the first case of FDCS of tonsil.

The lack of awareness of this entity causes a high rate of misdiagnoses in extranodal cases, especially in the head and neck region. Head and neck FDCS have occurred in soft palate, retromolar trigone, nasopharynx and parapharyngeal space, parotid gland. FDCS of tonsil is extremely rare and so far only a total of 49 cases (35 cases in the English literature and 12 in the Chinese literature) were reported (see Tab. I). To date, no such cases occurring in Uganda are described in the literature. Therefore, we herein present our case, with a review of the pertinent literature.

**Case report**

A 60-year-old Ugandan female patient presented to Gulu Regional Referral Hospital (north Uganda) with a fungating, painless left tonsillar tumour of 6-year-duration. The mass had caused difficulty in swallowing for the
### Tab. I. Summary data of FCDS of the tonsil reported in the English literature. Data of cases reported in the Chinese literature were retrieved from the article by Lu et al. 26.

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<th>Ref</th>
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<th>Pt's age</th>
<th>Side</th>
<th>Max size (cm)</th>
<th>Metastases at diagnosis</th>
<th>PO treatment</th>
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<td>76</td>
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<tr>
<td>8</td>
<td>F</td>
<td>48</td>
<td>L</td>
<td>3.5</td>
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<td>Radical neck dissection</td>
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<td>17</td>
<td>M</td>
<td>45</td>
<td>R</td>
<td>NA</td>
<td>/</td>
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<td>NA</td>
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<tr>
<td>18</td>
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<td>44</td>
<td>L</td>
<td>1.5</td>
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<td>NA</td>
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<td>37</td>
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<td>18</td>
<td>R&amp;L</td>
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<td>9</td>
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<td>27</td>
<td>R</td>
<td>4</td>
<td>1 cervical LN</td>
<td>Neck dissection + RT</td>
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<td>M</td>
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<td>/</td>
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<td>F</td>
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<td>NA</td>
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<td></td>
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<td>77</td>
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<td>14</td>
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<td>5</td>
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<td>RT</td>
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<tr>
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<td>R</td>
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<td>/</td>
<td>CHT</td>
<td>NA</td>
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continues
last three months. Intraoral examination revealed a red mass with an irregular surface over the left tonsil. Tonsil of the opposite side was normal and a neck examination revealed no enlarged lymph nodes. General systemic examination was unrevealing. The patient underwent tonsillectomy under general anaesthesia.

Grossly, the resected solid mass measured 6 cm in diameter and showed a bosselated outer surface. Cut section revealed a whitish firm tumour with numerous tiny areas of haemorrhage and smooth surface.

Microscopically, the tumour was situated underneath the squamous epithelial lining of the tonsil (Fig. 1a). It had a diffuse growth pattern and pushing borders. There were focal storiform areas arranged in centripetal whorls reminiscent of meningioma, and focal multinucleate giant cells. Individual cells tended to be ovoid, with central nuclei, slightly eosinophilic, fibrillary cytoplasm and indistinct cell borders. The nuclei had fine chromatin, small but distinct nucleoli and a delicate nuclear membrane. Small lymphocytes were scattered throughout the tumour and often clustered around blood vessels (Fig. 1b-c). On immunohistochemistry, the tumour cells expressed strong membrane positivity for all the three markers specific for follicular dendritic cell differentiation e.g. CD21, CD35 and CD23 (Fig. 1a) and for EGFR (Fig. 2a). Nuclear positivity for p53 was also observed (Fig. 2b). It was negative for cytokeratins, CD45, S-100, CD68, TTF1 and EBV-latent membrane protein-1. The Ki-67 labeling index was about 25% (Fig. 2c). Patient received no further treatment and was subsequently lost to follow-up.

Discussion

We reviewed all of the 49 published reports of tonsil FDCS (Tab. I). Twenty-four cases occurred in women and 24 in men (gender not available in 1 instance). Patient’s age at diagnosis ranged from 80 to 18 years (mean: 47.8 yrs). FDCS in women tended to occur at younger age (mean: 44.5 yrs) than in men (mean: 49.4 yrs). The age difference between males and females is not statistically significant. A total of 20 tumours were located in the left tonsil, while 19 were located in the right tonsil; 2 were bilateral. The affected tonsil was not reported in eight cases. Tumour size ranged from 0.8 to 5 cm in maximum dimension (mean 2.9 cm). In approximately half of the instances (24 cases) tonsillectomy was the only treatment. Seven had additional radical neck dissection. Fourteen cases received pre- or postoperative radiotherapy, including five cases with concomitant neck dissection. Two cases received combined chemotherapy and radiotherapy and 7 chemotherapy alone. One patient received no treatment at all. Six patients (12.2%) presented with metastatic disease at initial diagnosis, all localised in the cervical lymph nodes. Follow up information was present from 4 to 180 months with an average of 38.2 months. A minimum follow-up of 12 months was available for 28 patients. Of them, 25 cases were alive with no evidence of disease after this period of time, indicating a rate of approximately 50% disease-free survivals at 1-year.

According to Pang et al, oropharyngeal FDCS are significantly more likely to present with regional metastases compared to non-oropharyngeal lesions, including FDCS of the

<table>
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<th>Pt’s age</th>
<th>Side</th>
<th>Max size (cm)</th>
<th>Metastases at diagnosis</th>
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<th>Tobacco use</th>
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<td>38</td>
<td>F</td>
<td>69</td>
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<td>na</td>
<td>/</td>
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<td>Lung and ilar LN metastases (96) AWD (108)</td>
</tr>
<tr>
<td>31</td>
<td>M</td>
<td>63</td>
<td>L</td>
<td>4.2</td>
<td>/</td>
<td>No treatment</td>
<td>NA</td>
<td>AWD (8)</td>
</tr>
<tr>
<td>32</td>
<td>F</td>
<td>52</td>
<td>R</td>
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<td>/</td>
<td>CHT</td>
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<tr>
<td>33</td>
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<td>/</td>
<td>RT</td>
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<tr>
<td>35</td>
<td>F</td>
<td>54</td>
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</tr>
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<td>36</td>
<td>M</td>
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Wang (26) M 80 R 4.6 / None NA NED (24)
Wu (26) F 55 L NA / None NA NED (4)
Yang (26) F 49 R 5 / CHT NA NED (22)
Yin (26) M 35 R 5 / None NA LN metastasis (12) bilateral neck dissection + RT NED (39)
Zhan (26) M 36 R NA / None NA NED (48)
Zhan (26) F 43 R 3 NA / None NA NA

Abbreviations: NA: not available; M: male; F: female; CHT: chemotherapy; LN: lymph node; NED: no evidence of disease; DOD: died of disease; REC: recurrence; AWD: alive with disease
cervical lymph nodes. However, this does not hold true for the palatine tonsil, as only 6 of 49 cases (12.2%) showed local metastasis to lymph nodes at initial diagnosis. Presently, surgery with no adjuvant treatment is considered to be the standard for FDCS treatment. When metastasis to regional lymph nodes is suspected on imaging studies, a neck dissection is recommended. In the current case, dissection was not performed because no
findings suggestive of metastasis to the cervical lymph nodes were observed intraoperatively.

Adjuvant radiotherapy or chemotherapy can, however, be applied to cases with adverse pathologic features and in cases of advanced or incompletely resected lesions. Complete responses to CHOP are rare, and the benefits of this regimen may derive primarily from doxorubicin, one of the most broadly active agents against sarcoma in general. Although the pathogenesis of this neoplasm remains unknown, in 10% to 20% of cases it occurs in association with Castleman’s disease of the hyaline-vascular type. Epidermal growth factor receptor (EGFR) expression has been investigated as another shared feature of both entities. In addition, overexpression of the p53 protein is noted in FDCS. The present case showed diffuse hyperexpression of both EGFR and p53. Small molecule tyrosine kinase inhibitors and monoclonal antibodies are currently used to block EGFR activity and may prove of therapeutical interest in FCDS.

Reactive proliferations of FDCs are found in human immunodeficiency virus (HIV)-associated lymphadenopathy. Recent research studies have demonstrated that HIV infection may play a role in the formation of FDCS, via previous exposure to the Epstein Barr Virus (EBV). The association with EBV infection is acknowledged in the setting of the inflammatory pseudo-tumour-like variant of FDCS. However, no cases of extranodal FDCS of the head and neck region have been associated with EBV. Heavy tobacco smoke habit was reported in some of the tonsillar (Tab. I) and extratonsillar cases. According to the fourth edition of the WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, most FDCS show low or intermediate-grade malignancy. High-grade FDCS can be identified by large tumour size (greater than 6 cm), intraabdominal location, significant cytological atypia, extensive coagulative necrosis, and high proliferative index. The present case was notable for the long duration of the disease (6 years) before the patient sought medical attention, as she lived in a poor rural area. Such a slow growth indicates a low-grade malignant potential of FDCS. Additionally, recurrences may be delayed for many years (Tab. I). Owing to the short-term follow-up periods in the reported cases, the local recurrence and metastatic rates might be underestimated. 72.5 months was the mean period for local or distant recurrences in this reviewed series of tonsil FDCS, with the longest disease-free interval of 180 months.

FDCS are rare tumours that have been recognised only recently. The rarity of these tumours makes them difficult to accurately diagnose and treat, and they are often mistaken as non-Hodgkin lymphoma or other lymphoproliferative disorders. Immunohistochemistry may help in the diagnosis of FDCS (Tab. II). Of interest, despite coexpression of a leukocyte common antigen (CD45), and CD15, a monocyte common antigen in normal FDCs, in our experience and in all, but two reported cases of sarcomatous transformation, CD45 was negative. Revision of FDCS immunohistochemical literature data cannot confirm Grogg et al.’s impression that tu-

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Abbreviations: NA: not available; MSA: muscle specific actin; NSE: neuron specific enolase.
mours displaying more morphological and phenotypical (CD68+, S-100+) histiocytic differentiation may be associated with more aggressive behaviour, more akin to the behaviour of histiocytic sarcoma (Tab. II).

Conclusions

FDCS should be included in the differential diagnosis for any tonsillar mass, as an increasingly high number of cases are being added to the literature, thanks to increased awareness of this entity and more sensitive diagnostic tool. Our case shows that this tumour can also affect African patients. This should not come as a surprise in the light of a possible association of FDCS with viral infections, as the population of north Uganda is still plagued by AIDS and EBV infections.

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Is it possible to determine the origin of cyst in empty thyroid bed in patient with lingual thyroid?

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Key words
lingual thyroid • cyst in empty thyroid bed • Tc-99m-pertechnetate scintigraphy • iodine-131 scintigraphy • SPECT/CT

Summary
Some patients with ectopic thyroid gland or athyreotic patients have one or more cysts in empty thyroid bed. The origin of these cysts is uncertain. We present the patient with lingual thyroid gland and small cyst in empty thyroid bed featuring the diagnostic algorithm used and discussing the possible etiologic scenarios.

Introduction
An ectopic thyroid tissue refers to all cases in which the thyroid gland tissue is present at a location other than its usual pretracheal position in the lower neck. It is an uncommon embryological anomaly resulting from no or abnormal migration of the thyroid cells from their origin in the floor of the primitive pharynx to its usual cervical location. Lingual thyroid, located at the base of the tongue in the midline, is the most commonly found ectopic thyroid tissue and accounts for 90% of the reported cases although ectopic thyroid tissue may be found anywhere along the path of descent of the developing thyroid primordium, such as sublingual, cervical, mediastinal, intratoracic and subdiaphragmatic location. In most cases the ectopic thyroid is the only functioning thyroid tissue.

The presence of cysts in cervical thyroid area have been described in patients with ectopic thyroid tissue or in athyreotic patients. Etiology of this additional developmental anomaly is uncertain. They might be due to the persistence of the ultimobranchial bodies as a cystic structure or part of the thyroid-forming material, which may migrate along the normal pathway of the usual course of the thyroglossal duct, giving rise to cell residues within the empty thyroid area.

We report one such patient with lingual thyroid and cyst in empty thyroid bed and attempt to elucidate the origin of this cyst.

Case report
An asymptomatic 32-year-old woman was referred to our outpatient clinic for further investigation after her routine laboratory data showed evidence of subclinical hypothyroidism: T3 was 1,3 nmol/L (normal range = 1,3-3,6nmol/L), T4 was 72 nmol/L (normal range = 58-161 nmol/L) and TSH was 10,5 mIU/L (normal range = 0,3-3,16m IU/L). On clinical examination the thyroid gland was not palpable in its usual cervical location and there was no other palpable mass on the neck. Ultrasonography revealed empty thyroid bed with small cyst on the right side that measured 7 x 6 x 7 mm (Fig. 1A- transverse and 1B- longitudinal sonogram). Detailed ultrasound examination of other cervical regions didn’t disclose any additional abnormality but coronal and sagittal view through the floor of the mouth, revealed hypoechogenic echostructure at the base of the tongue that measured 26x24x15 mm (Fig. 1C-coronal and 1D-sagittal sonogram). To confirm that this hypoechogenic structure corresponded to lingual thyroid, patient was referred to Tc-99m-pertechnetate scintigraphy (Fig. 2A-anterior and B-right lateral view),
Fig. 1. Cyst in empty thyroid bed and lingual thyroid. Transverse (A) and longitudinal sonogram (B) shows cyst on the right side in empty thyroid bed measuring 7x6x7 mm. Posterior coronal and midline sagittal sonogram from the submental region through the mouth floor (C and D) shows spherically hypoechoic mass at the base of the tongue that measures 26x24x15 mm and corresponds to the lingual thyroid.
which showed the uptake of radiotracer in the mouth cavity and absence of radiotracer activity in the normal cervical thyroid region. Iodine-131 scintigraphy (Fig. 2C) of the head, neck, thorax and abdomen also showed only a single focus of activity in the mouth projection without any activity in the usual cervical thyroid region without any other ectopic activity. Small activity of excreted I-131 is visible in the stomach, bowels and bladder. Iodine-131 SPECT/CT (low dose) (D) localizes this ectopic thyroid tissue at the base of the tongue, confirming the existence of lingual thyroid.
and without any other ectopic activity. The Iodine-131 SPECT/CT (low dose) (Fig. 2D) localized this ectopic thyroid tissue at the base of the tongue, confirming the existence of lingual thyroid. The sonographically guided fine-needle aspiration cytology of the small cyst in the thyroid bed yielded the translucent viscous content. In cytologically smear (Fig. 3.) there were macrophages and precipitate without follicular or parafollicular cells, but in punctate both specific markers of these cells (thyroglobulin and calcitonin) were positive. This provided the circumstantial evidences of existence of follicular and parafollicular cells in the cyst. In aspirate the thyroglobulin was 201.7 ng and calcitonin was 213.9 pg. Thyroglobulin and calcitonin in serum specimen were 2.8 ng/ml (reference value = < 40 ng/ml) and 3.5 pg/ml (reference value = < 10 pg/ml) respectively. Thyroxin therapy was prescribed and on control examination three months later TSH was normal.

Discussion

The thyroid gland has a dual embryonic origin and produces two different hormones by two different cell types: the thyroid hormones by thyroid follicular cells and calcitonin by parafollicular or C cells, respectively. The most abundant cells, thyroid follicular cells, thyrocytes also produces a thyroglobulin. Thyrocytes arise from the embryonic endoderm as a thickening in the floor of the primitive pharynx, whereas C cells precursors migrate from the neural crest bilaterally to the fourth and fifth pharyngeal pouches and become localized in the ultimobranchial bodies. Ultimobranchial bodies are a pair of pharyngeal pouches and become localized in the ultimo-tracheal region, two lateral anlagen, the ultimobranchial bodies. In the seventh week of embryonic development, the thyroid reaches its final position in front of the trachea anterior to the developing second, third and fourth tracheal rings. At that time it connects with the two lateral anlagen, the ultimobranchial bodies. In the coupling process both the thyroid anlage and the ultimobranchial bodies disappear as individual structures, and the cells contained in them disperse in the structure of the thyroid gland. Follicular cells from median anlagen continue to organize in the thyroid follicles, whereas the calcitonin producing C cells remain scatter within the interfollicular space, mostly in a parafollicular position.

The small cyst on the right side of the empty thyroid bed in our patient is not uncommon finding in patients with ectopic thyroid or athyreosis, but its origin is uncertain. For example, in 57 children with congenital hypothyroidism, 42 with ectopic thyroid tissue and 15 with athyreosis, cysts in empty thyroid bed were found in 39 patients (68% of cases), 29 of them with ectopic thyroid and 10 of them with athyreosis. All cysts were located in the empty thyroid area, 57% in the left and 43% in the right side, closer to the midline in all cases. Those patients had either single cyst (16 patients) or multiple cysts (23 patients) and the cysts were bilateral in 17 of the 39 patients. Most of the cysts were vertically oval or round, with a size ranging in diameter from 2-21 mm². The authors speculated that these cysts might be due to the persistence of the ultimobranchial bodies as a cystic structure or a part of the thyroid-forming material, which may migrate along the normal pathway of the usual course of the thyroglossal duct, giving rise to cell residues within the empty thyroid bed. The exact nature of these cysts couldn’t be established by histological examination because histological verification was unjustified in these patients. Pathohistology examination of four out of five autopsies specimen of cysts in thyroid bed, in the region of the upper parathyroids in patients with lingual thyroid, revealed both follicular and parafollicular-C cells. Since the ultimobranchial body derives from the fourth and fifth branchial pouches and descends with parathyroid IV to fuses with the thyroid, while parathyroid IV remains as the upper parathyroid closely applied to the thyroid lobe, and because these unusual cystic structures in these patients were found immediately adjacent to the upper parathyroid in absence of normally descended thyroglossal duct-derived tissue, the authors concluded that these structures derive from the ultimobranchial tissues and that the ultimobranchial body contributes both C cells and follicular cells of the thyroid in

Fig. 3. Fine needle aspiration cytology of small cyst in empty thyroid bed in patient with lingual thyroid shows only macrophages without follicular or parafollicular cells (May Grunwald Giemsa stain, magnification x 400).
This thesis of ultimobranchial origin of follicular and parafollicular cells is supported by the reports of patients with ectopic thyroid presenting as a submandibular mass without detectable thyroid tissue in the normal median position. and ectopic thyroid tissue located in the carotid space with orthotopic thyroid gland. At last, the remnants of ultimobranchial bodies in normal thyroids are usually referred by pathologists as solid cell nests. It is now widely accepted that solid cell nests and so-called "mixed" follicles are indeed ultimobranchial body remnants. Solid cell nests are composed of main cells and C cells. It has been suggested that main cells might be pluripotent cells contributing to the histogenesis of C cells and follicular cells, as well as to the formation of certain thyroid tumors. According to these, the cysts in thyroid bed must represent the cystic degeneration of the remnants of the ultimobranchial bodies, which contains both, follicular and parafollicular cells.

In contrast to these theses, there are evidences of existence of parafollicular cells in lingual thyroids. Pathohistology examination of six lingual thyroids confirmed coexistence of follicular and parafollicular cells. The authors speculated that primordial thyroid follicular cells contains pluripotent stem cells from which the follicular and parafollicular cells can derive. According to the authors, another possibility is that some non-stem cells can transdifferentiate to calcitonin producing cells. These findings suggest that the ultimobranchial bodies are not the only source of calcitonin-producing cells in humans. The corroborated finding is the detection of medullary thyroid carcinoma in a lingual thyroid. One can conclude that if only a few clusters of these multipotent cells from lingual thyroid migrate to the usual pretracheal thyroid position, and if they undergone cystic degeneration, the result may be cyst in empty thyroid bed.

Theoretically, there is also the possibility that follicular cells in cyst derived from medial anlage and parafollicular cells from lateral anlage.

Conclusions

We present the patient with two developmental anomaly of the thyroid tissue. Beside ectopic, lingual thyroid, the patient had cyst in empty thyroid bed. Although we provided circumstantial evidences of the presence of both follicular and parafollicular cells in the cyst, this does not suffice to determine the cyst origin and this issue remains the controversial one.

References

Errata Corrige

Pubblichiamo la seguente relazione erroneamente omessa da PATHOLOGICA 2016;108:192

7° Congresso Triennale di Anatomia Patologica Siapec-IAP 2016

Mercoledì, 23 novembre 2016
Aula Maestrale – 13:30 - 17:30

Qualità e sicurezza

Moderatori: Paolo Dalla Palma (Trento) - Giuseppe Santeusanio (Roma)

RIORGANIZZAZIONE TERRITORIALE DEI LABORATORI DI ANATOMIA PATOLOGICA: PRO E CONTRO

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La medicina di Laboratorio oggi è percorsa da forti tensioni al cambiamento. Le opportunità offerte dalla rapida evoluzione tecnologica dei sistemi diagnostici e l’aumento dei costi della diagnostica, in un contesto di crescenti difficoltà di bilancio della sanità, hanno causato una tendenza all’affermarsi di nuovi modelli organizzativi. Nel percorso diagnostico-terapeutico il ruolo dell’Anatomia Patologica risulta centrale in quanto con un atto medico, rappresentato dalla diagnosi citologico-anatomopatologica, si finalizza una sequenza di procedure tecno-cognitive tese all’esame di organi o campioni di organi, inserendosi come elemento fondamentale e spesso decisivo nell’inquadramento clinico del paziente, indirizzandone le scelte terapeutiche e fornendo anche informazioni con rilevanti implicazioni prognostiche. L’introduzione di terapie di alto costo, specialmente per malattie oncologiche, la cui prescrizione è dipendente da specifica diagnosi anatomo-patologica, ha portato ad un incremento della richiesta diagnostica specialistica ed alla necessità di applicazione di procedure avanzate e di formazione di personale qualificato per test specializzati, quali, ad esempio, quelli molecolari. A garanzia dell’appropriatezza, dell’efficacia e della compatibilità economica delle prestazioni sanitarie, il piano oncologico nazionale ha sottolineato la necessità di centralizzare presso unità operative di Anatomia Patologica la diagnosi, che per le strutture, pubbliche e private, che rende necessario il superamento della frammentazione per garantire la qualità delle prestazioni. Riferendosi a laboratori di base e/o con sezioni specializzate, l’Accordo propone, come soglia minima per l’accreditamento di un laboratorio, un volume di attività di 200.000 esami di laboratorio complessivamente erogati annui, prodotti in sede e non tramite service, ma per i laboratori specializzati, come l’anatomia patologica, la genetica medica, la microbiologia, prevede la necessità di “considerazioni diverse e più articolate. A questo riguardo il DM 70 del 2 aprile 2015 “Regolamento recante definizione degli standard qualitativi, strutturali, tecnologici e quantitativi relativi all’assistenza ospedaliera” stabilisce le basi di utenza minimi e massimi, che per le strutture di Anatomia Patologica sono un numero di abitanti da 150.000 a 300.000. Quasi nello stesso tempo, tuttavia, una nota del Ministero della Salute del 16 aprile 2015 registra un’applicazione delle norme molto variabile a livello regionale, con “difficolta operative e modalità inique di attuazione di quanto previsto dall’Accordo 61 CSR” in numerose realtà. In effetti la riorganizzazione territoriale delle strutture di Anatomia Patologica, seppur pianificata nella maggior parte dei casi secondo un sistema di architettura “hub e spoke”, viene attuato in maniera molto diversa nelle svariate realtà.
regionali, con risultati contrastanti. Fra le soluzioni adottate: le aree vaste, come per prime in Emilia Romagna e Toscana, gli accorpamenti tra unità operative della stessa azienda ospedaliera, la creazione di dipartimenti interaziendali specialistici, la creazione di reti territoriali per un network collaborativo. Ciascuna delle soluzioni adottate presenta punti di forza e punti di debolezza: la loro applicazione deve tenere conto delle singole realtà in essere per poter conseguire i vantaggi previsti (rimodulazione delle dotazioni di personale in modo coerente con l’efficienza/efficacia dell’attività diagnostica, appropriato dimensionamento delle dotazioni tecnologiche, valorizzazione e promozione delle competenze, razionalizzazione della spesa). Sulla base di queste considerazioni di carattere generale verranno illustrati in dettaglio, anche partendo da esperienze concrete, gli aspetti positivi e negativi di questo processo di cambiamento, certamente positivo sul piano teorico, ma di difficile e complessa realizzazione.