Among all pathology fields, the analysis of melanocytic lesions has one of the highest rates of review for legal reasons, particularly regarding the distinction between nevus and melanoma (desmoplastic melanoma, nevoid melanoma, Spitz nevus vs. spitzoid melanoma, etc). Therefore, it follows that pathologists should pay special attention when dealing with such type of lesions. This lecture will emphasize a number of clinical, histological, and immunohistochemical features we believe are essential when evaluating such differential diagnosis. Furthermore, we want to stress the importance of examining the entire slide within the context of all available information in order not to miss the invisible gorilla in the slide.

Although we do not usually notice, our senses are very selective. At any given moment, there is a huge amount of information being processed from our retinas, cochleas, nose, etc. Just regarding our vision, the images from the entire retina are delivered to our occipital cortex but while processing those images, our brain selects which ones are “important”. As an example, if we start crossing a street and there is a car speeding fast to us, our brain focuses in that image to warn us of the danger while “erasing” the rest of the area captured by the retina.

Obviously, any selection method is very helpful if it really points to the dangerous/important image, but it may be counterproductive if our brain deletes other essential data. A famous study from the 70’s indicated that 50% of observers missed in a video the presence of an actress dressed up as a gorilla passing among a group of basketball players! And although we may consider that missing a gorilla in a basketball game is not very important, there may be other situations in which missing such “deleted” portions of information may be crucial. In an example closer to us, if a skin biopsy has a small nevus in the epidermis and our brain focuses on that area, we may miss the presence of metastatic breast carcinoma in the lymphatic vessels located in the deep reticular dermis!

In order to try to limit the effect of unconscious selection of data while signing out skin specimens, most pathologists stress the importance of having a systematic approach to sign out. In my case, I always look to the slide from the top (usually the stratum corneum) to the bottom (dermis/subcutaneous tissue) and from left to right. Such method forces me to at least “see” the entire slide (although it does not guarantee that I will “observe” it, as Sherlock Holmes would point out). Particularly helpful I think is the method that my mentor, Scott McNutt, used to tell us at the microscope, about “what to do when you see nothing on the slide” or what other authors have indicated as the study of “invisible dermatosis”. In essence, one should look at the stratum corneum to search for neutrophils, dermatophytes, bacteria, parakeratosis, ichthyosis, etc; then look at the stratum spinosum to determine if it is normal, thin, thick, if there is hypergranulosis of flat warts, if there is separation of the epidermis from the dermis (epidermolysis bullosa), amyloid deposition in the papillary dermis, and so on.
In addition to this systematic approach, we recommend, as others, to establish a preliminary histologic diagnosis without any clinical information. The alternative of examining the slides knowing the clinical information may limit ourselves to search for whatever diagnosis our clinical colleagues wanted us to rule out and thus miss something else important. Obviously, this “blinded” method of histologic analysis may result in egregious blunders in front of a colleague or a trainee (in more than one occasion I have suggested to our fellows that the features of the specimen were those of benign lichenoid keratosis just to find out that the patient had a disseminated eruption with flat-topped papules thus consistent with lichen planus or lichenoid drug reaction). However, it has the advantage that again forces us to examine the entire slide to try to gather all possible relevant information. As an opposite example, if we receive a specimen clinically diagnosed as “keratosis” but rather than limiting to the epithelial cells we examine the entire slide we may notice that there scattered cells with clear cytoplasm at the base of the epidermis, thus consistent with melanoma in situ with extensive lichenoid infiltrate!

Regarding particular situations to which we pay special attention during our sign-out we may classify them in three categories:

- **Clinical features**: After examining the slide, we always try to correlate with the clinical data.
  - Age: Solar elastosis in young individuals may be a sign of xeroderma pigmentosum or, more likely, a switched specimen.
  - Location: Prominent sebaceous glands and numerous hair follicles are typical of face biopsies while thick stratum corneum should be seen in acral biopsies. In addition to possibly being helpful for the diagnosis (some entities are more common in some anatomic areas, such as desmoplastic melanoma in sun-damaged skin) determination of the anatomic area likely to have been biopsied may help us detect a switched specimen.
  - When reviewing outside material, detection of a discordance between the time of the biopsy and the time of review. This is particularly important when evaluating old “nevi” because the reasons for review may be the current diagnosis of a recurrence or a metastasis.

- **Histologic features**:
  - Caution with dermal surgical scars when we do not know what the original diagnosis was. Always keep in mind the possibility of desmoplastic melanoma, try to detect perineural invasion (both in melanoma and carcinoma), and stromal tumors (such as residual dermatofibrosarcoma protuberans).
  - In standard, straightforward nevi, we should be careful in those cases in which the clinical information is “Changing mole”. In such instances we should look for a reason to explain such change: focus of melanoma, ruptured folliculitis, trauma, etc. If necessary, it may be helpful to examine additional sections from the block.
  - Presence of hyperkeratosis and parakeratosis in a melanocytic lesion may point to previous trauma. This possibility should then be considered in lesions that show focal pagetoid upward migration limited to such areas of parakeratosis before establishing the diagnosis of melanoma. In a related note, lesions that show prominent single-cell growth with focal pagetoid upward migration limited to the area with dermal fibrosis are likely “recurrent nevus/pseudomelanoma” as seen in partially biopsied melanocytic lesions.
In adults, close relationship of melanocytes with solar elastosis in the dermis usually points to a long-standing lesion. Thus, such finding is more consistent with a nevus and is very unlikely to be a metastasis or rapidly growing melanoma.

While most melanoma lesions seen in the dermis or subcutaneous tissue and lacking connection with the overlying epidermis are metastatic melanoma, it is always necessary to search for the presence of a benign melanocytic component (associated nevus) in the adjacent dermis/subcutaneous tissue. A relatively common pitfall is the diagnosis of metastatic melanoma in a dermal proliferation of pigmented melanoma cells if the associated area of blue nevus (this indicating that it is a primary lesion) is missed during the review of the slides.

In sentinel lymph nodes, detection of melanoma metastasis is associated with an impaired prognosis. However, not all melanocytes seen in the lymph nodes are melanoma cells (up to 10% of patients with melanoma will show benign, nodal nevi in their sentinel lymph nodes). Apart from the cytologic characteristics of such benign melanocytes (small nucleoli, absence of mitotic figures), their location within the node is essential for the diagnosis. Most nodal nevi will be present in the fibrous capsule. In contrast most metastases will occupy the subcapsular sinus. However, a very rare instance is the detection of melanoma cells in lymphatic spaces within the fibrous capsule. For those cases immunohistochemistry (D2-40, CD31, or CD34) may be needed to detect a rim of endothelial cells around the melanocytes.

In sentinel lymph nodes containing nodal nevi, they may be occasionally intraparenchymal. However, such cases usually show an evident area of involvement of the fibrous capsule. Thus, when seeing melanocytes both within the capsule and the immediately adjacent subcapsular sinus, they usually correspond to nodal nevus (in the rare occasions in which nodal nevus and melanoma co-exist in the same lymph node, they usually occupy different areas of the node).

**Immunohistochemical features:** In our opinion, immunohistochemistry provides additional information that is a very important adjunct tool in the diagnosis of melanocytic lesions:

- **S100 protein** may be lost if the specimen is under- or overfixed or if it has been previously frozen. As sometimes S100 protein is the only “melanocytic” marker expressed in some melanomas (mostly desmoplastic melanomas), such “false” negative may result in an important misdiagnosis. Furthermore, we have noticed that the epitope expressed in melanocytes is sometimes more labile than that expressed in other cells (Schwann cells, neurons) so therefore it is essential (when available) to check if normal melanocytes (in the epidermis or adnexa) are positive for this marker in the slide, before definitely considering that a tumor is S100 “negative”. Another possible pitfall related to S100 protein is that it can be expressed in other neoplasms. In particular, Paget disease of the breast may express such marker, so keratins should be also included in a panel when evaluating such differential diagnosis.

- **Melanomas**, as it happens with other neoplasms, not always confirm to the expected pattern of expression. They may lack all melanocytic markers (very rarely), express low molecular weight keratin, estrogen receptors, CD68, etc.

- **MART1** is a very specific marker for melanocytic differentiation. However, some macrophages (mostly those containing melanin pigment) may become positive after antigen retrieval. Such cases require careful examination of the cytologic details and
possibly use of other melanocytic markers (tyrosinase, HMB45). On the other hand, analysis of MART1 expression may be very important in the differential diagnosis of desmoplastic nevi and desmoplastic melanomas. Since the immense majority of benign melanocytes, when dealing with a melanocytic lesion (usually with a spindle cell morphology) that does not express MART1, it is likely to be desmoplastic/spindle cell melanoma.

- HMB45 is usually negative in the deep areas of standard nevi as well as in nodal nevi. However, if the antigen retrieval technique is very robust, such nevus cells may become positive thus suggesting a diagnosis of melanoma. Thus it is essential that the pathologist be used to the type of intensity that his/her immunolaboratory routinely has in HMB45 slides.
- MIB1 is very helpful since the immense majority of benign melanocytic lesions show very low (less than 5%) proliferation rate at the deep areas of the lesion. In cases with significant lymphoid infiltrate, a double immunostudy (e.g., with MIB1 and anti-MART1) may help detecting if the proliferating cells are melanocytes.

In summary, we believe that the analysis of melanocytic lesions (as well as any other pathology specimen) should be performed in a systematic way, whichever one is selected by the pathologist. Only by always considering the possibly pitfalls/diagnostic problems will we avoid “missing that invisible gorilla (melanoma)” in our slides.

References:

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