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Review Article

Pitfalls in Diagnostic Hematopathology: Part I

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Abstract: Pitfalls in diagnostic hematopathology are underestimated and underreported. Major causes of diagnostic error in hematopathology include: 1) inadequate material; 2) inadequate workup; 3) inadequate clinical correlation; 4) over or under interpretation; 5) challenges in hematopathology. In the first part of this review series, I will focus on the pitfalls in diagnosing and differentiating chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma, and discuss the strategies to avoid potential diagnostic errors based on my personal experience.
Key Words: Hematopathology, pitfall, mantle cell lymphoma, chronic lymphocytic leukemia/small lymphocytic lymphoma

Introduction

Pitfall is a popular term in the medical community. As defined by *The American Heritage® Dictionary of the English Language* (Fourth Edition, 2000), "pitfall" is an unapparent source of trouble or danger; or a hidden hazard. A *pitfall* in pathology is a *diagnostic risk* that pathologists should be *aware of* and *avoid*. When searching the PubMed using "pitfall in pathology", there were 759 hits as of February 2008. Using "pitfalls in pathology", 1931 hits were found. Interestingly, most of these pitfalls were related to cytopathology. 739 hits were identified using keywords "pitfall in cytopathology", and 1781 hits were found with "pitfalls in cytopathology". When either "pitfall" or "pitfalls in hematopathology" was used, only 8 hits were encountered, much fewer than I expected. *Does it mean that there are fewer pitfalls in hematopathology?* It is apparently not, and the *pitfalls* in hematopathology are *under* reported.

Some of the most common *pitfalls* encountered in our daily practice of hematopathology are summarized in **Table 1**. In this article, I will briefly review the common causes of diagnostic errors in hematopathology, and discuss in length the pitfalls in the diagnosis and differential diagnosis of chronic lymphocytic leukemia/ small lymphocytic lymphoma (CLL/SLL) and mantle cell lymphoma (MCL) and how to avoid the potential diagnostic errors.

Common Causes of Diagnostic Error in Hematopathology

Why do we pathologists make mistakes? Pathologists are humans – humans err. To decrease or prevent diagnostic errors, we have to understand the factors that may cause mistakes. The major causes that could lead to diagnostic errors in hematopathology include the followings.

Inadequate Material

This includes insufficient diagnostic specimens and sampling errors. If insufficient material is submitted, we do not have the substantial basis to evaluate. On the other hand, if the specimen is not representative of the lesion, the diagnostic features of the lesion will not be present and the incorrect diagnosis could be potentially made.

Inadequate Workup

Even with adequate diagnostic materials, potential diagnostic mistakes can occur even with adequate diagnostic material if initial

Table 1 Common pitfalls in diagnostic hematopathology

Disease A	versus	Disease B
CLL/SLL		MCL
EBV Infection		Malignant lymphoma
Hodgkin lymphoma	Grey zone lymphoma	Diffuse large B-cell lymphoma
Diffuse large B-cell lymphoma	Grey zone lymphoma	Atypical Burkitt lymphoma
Hematogones		Precursor B cell ALL/LBL
Precursor T ALL/LBL		Thymoma

CLL/SLL, chronic lymphocytic leukemia/small lymphocytic lymphoma; MCL, mantle cell lymphoma; EBV, Epstein-Barr virus; ALL/LBL, acute lymphoblastic leukemia/lymphoblastic lymphoma

workup is inappropriate. Triage of fresh specimen for lymphoma workup is critical in hematopathology.

Inadequate Clinical Information

Although pathology is the most objective specialty in medicine, a prepared mind is always favored. If we do not know what we are looking for, neither can we determine the way to look for it, the way to exclude it, and the criteria to commit it. For some cases, we can never make the right diagnosis without the clinical information.

Over or Under Interpretation

Pathologists are subjective. Pathologists are also judges based on the evidence provided by the specimen. However, due to the variations in training, knowledge and personal experience, pathologists sometimes do not agree among themselves. Using their subjective judgments, they may draw different conclusions on the same objective specimen. If we are not familiar with the typical morphology, atypical variants and its mimics, we may either over interpret or under diagnose a lesion, resulting in serious consequences.

Challenges in Hematopathology

We have to admit that one of the most important causes of misdiagnosis in hematopathology is the diagnostic challenges. Although hematopathology may have seen the most innovative diagnostic advances (such as flow cytometry, cytogenetics and molecular diagnostics) among all the pathology subspecialties, we still have little understanding on peripheral T-cell lymphomas, myelodysplastic syndrome, or even diffuse large B-cell lymphomas. Although there are successful examples of application of gene array and proteomics to the classification of leukemia and lymphoma [1-5], these assays are still far from our daily clinical practice.

Diagnostic Features of CLL/SLL and MCL

CLL/SLL and MCL are both B-cell lymphomas of small cell type (Kiel classification). In addition, both CLL/SLL and MCL aberrantly express CD5, a common T-cell marker. CD5 is expressed on two subsets of normal B-cells, näive (or virgin) B-cells that have not yet been exposed to antigens, and memory B-cells that can be activated into immunoblasts when they encounter the same antigen they contacted before. Since the former have never met antigen, they have not experienced the germinal center clonal selection process, and thus do not harbor somatic mutations of the immunoglobulin heavy chain variable (*IgHv*) region. In contrast, the latter have somatic mutations in the *IgHv* region. It is believed that CLL/SLL can be derived from both näive B-cells and memory B-cells [6]. Therefore, two subsets of CLL/SLL are identified based on whether or not there are somatic mutations in the IgHv regions. Studies have proved that this subset classification is clinically relevant because CLL/SLL derived from memory B-cells (*IgHv* mutated) have a much better prognosis (median survival 109 months) than those from näive B-cells (*IgHv* unmutated) (median survival 293 months) (see Table 2) [7-10]. It is currently believed that MCL derives from the mantle zone B-cells that are composed of CD5+ memory B-cells [11]. However, MCL has a much poorer prognosis (median survival 3-5 years) than their CLL/SLL counterparts. Owing to the different clinical courses, CLL/SLL and MCL are managed quite differently. Therefore, differentiating CLL/SLL from MCL is critically important.

In typical cases, CLL/SLL can be easily differentiated from MCL based on morphology alone. CLL/SLL usually infiltrates lymph node

Table 2 Two subtypes of CLL/SLL

Features	Naive B-cell	Memory B-cell
Germinal center exposure	No	Yes
Hypermutation of *IgHv*	No	Yes
CD38 expression	Often positive	Rarely positive
ZAP70 expression	Frequently positive	Rarely positive
AID mRNA expression	High	Low
Lipoprotein lipase A	High	Low
ADAM29	High	Low
13 microRNAs overexpression	Yes	No
Median survival (months)	109	293
Prognosis	Poor	Favorable

in a diffuse pattern, although it may appear vaguely nodular under the low power (**Figure 1A**). Under the intermediate power, they are actually pseudo nodules (**Figure 1B**). There is no fibrous septum or vessels between the pseudo nodules. Under the high power, those pseudo nodules are indeed proliferation centers with increased numbers of paraimmunoblasts that have more abundant cytoplasm and a single centrally located prominent nucleolus, and thus the nuclei of the lymphoma cells are far apart, giving rise to a lighter color on the Hematoxylin-Eosin stained sections (**Figure 1C**). Generally, CLL/SLL pseudo nodules are composed of a pleomorphic population of lymphoid cells ranging from small to medium-sized and having regular nuclear contours. On the other hand, MCL can infiltrate the lymph node in diffuse, nodular or more commonly mantle zone patterns (**Figure 2**). When in nodular patterns, MCL are true nodules with fibrous septum or proliferating blood vessels separating each individual neoplastic nodule (**Figure 2A**). Furthermore, the nodules are composed of a monomorphic population of small to medium-sized lymphoid cells with cleaved, folded nuclei and irregular nuclear contours (**Figure 2D**). The nuclei are usually packed closely together without conspicuous cytoplasmic membrane. The cells sometimes appear three-dimensional, appearing like exploding fireworks jumping into your eyes (**Figure 2D**). Large cells with prominent nucleoli are almost never identified in MCL except in the blastoid variant. However, mitotic figure are often present.

MCL occasionally involves the peripheral blood, conferring a blastoid variant and has to be differentiated from CLL, which is more

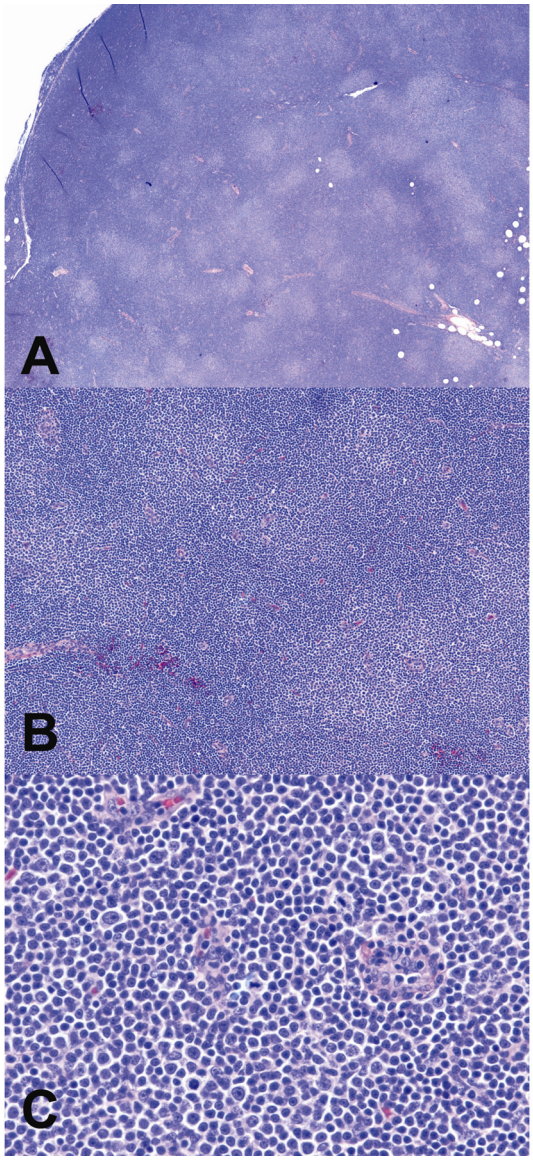

Figure 1 Classical histology of CLL/SLL under low power (**A**), medium power (**B**) and high power (**C**).

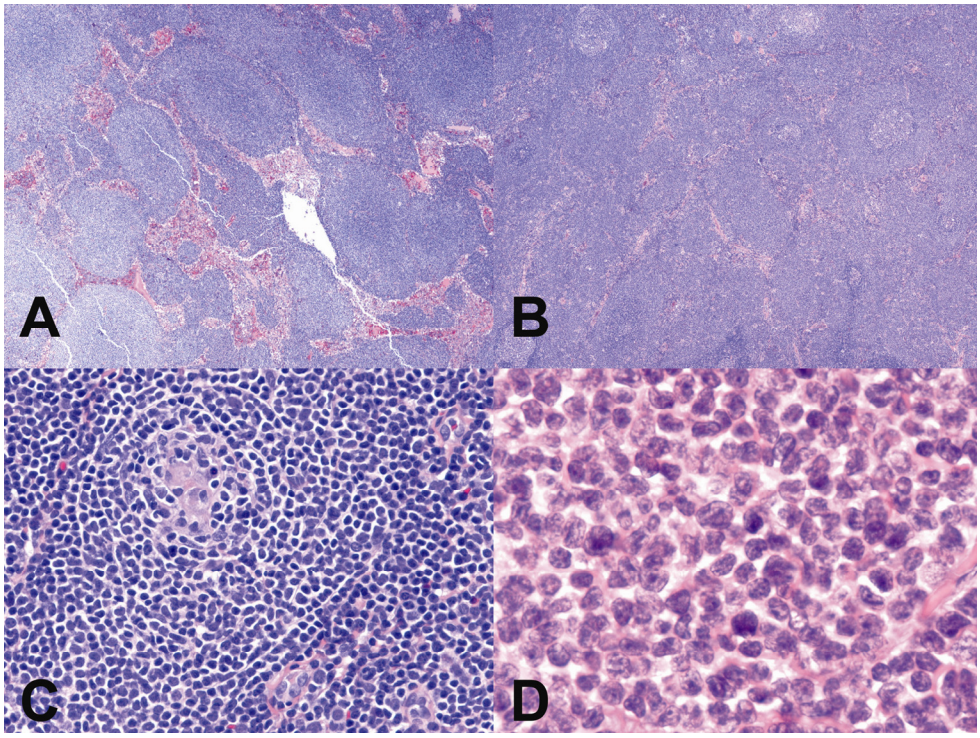

Figure 2 Histological features of MCL with a true nodular pattern (**A**) or a mantle zone pattern (**B**) with the remnant germinal center (**C**). Under high power, MCL cells are monotonous with scant cytoplasm and irregular nuclear contour (**D**).

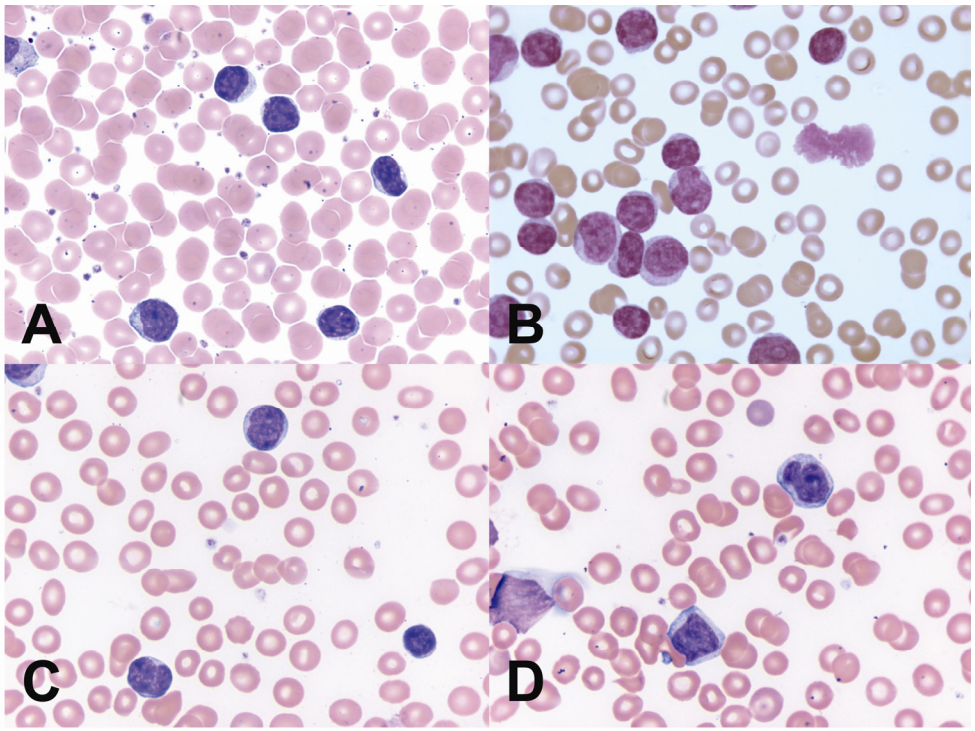

Figure 3 Comparison of the cytological features between CLL/SLL (**A** and **B**) and MCL (**C** and **D**).

common than MCL in involving peripheral blood and often presents with marked lymphocytosis (>10,000/dL). The neoplastic cells of CLL are small in size, with a rim of basophilic cytoplasm, round or slightly irregular nuclei and inconspicuous nucleoli with regular nuclear contour (**Figure 3A**). The chromatin is characteristically clumped or condensed with white areas in between, conferring a "soccer ball" appearance (**Figure 3B**), as described by one of my mentors Dr. Jay Hess. In contrast, MCL in peripheral blood usually show irregular nuclear contours, with cleaved or folded nuclei (**Figure 3C**). Sometimes the nuclei are squared-off, or even with pikes (**Figure 3D**). Lymphoma cells with abundant cytoplasm and prominent nuclei resembling prolymphocytes are not typically seen. Lymphoma cells similar to centroblasts or immunoblasts may be present in rare cases of blastoid variant of MCL or transformed MCL.

Diagnostic Pitfalls in CLL and MCL

The above cases are classical examples of CLL/SLL and MCL. In our daily practice, we may see CLL/SLL and MCL with atypical morphology. These cases can be diagnostic pitfalls. Fortunately, the development of various novel techniques makes our practice much easier than our preceding colleagues. With immunohistochemistry, flow cytometry, cytogenetics, and molecular assays, we are able to accurately diagnose >95% of CLL/SLL and MCL. The following immunological and genetic features are very useful in differentiating CLL/SLL from MCL (**Table 3**).

Although CLL/SLL and MCL share many of the mature B-cell markers immunophenotypically with aberrant expression of CD5, they have many different properties. By flow cytometry, CLL/SLL typically expresses low-density surface immunoglobulin, CD20, CD22 and CD11c, and is often negative for FMC7. On the other hand, MCL expresses high-density surface immunoglobulin, CD20, CD22, and is positive for FMC7. Most importantly, almost all MCLs produce cyclin D1 in the nuclei, which can be detected by immunohistochemistry (**Table 3**).

However, just like we can never say "*never*" or "*always*" in medicine, exceptional cases are present. These cases are the potential diagnostic pitfalls.

Pitfall 1: CD23-negative CLL/SLL

Although almost all the hematopathology books may state that CLL/SLL is CD5+ and CD23+; whereas MCL is CD5+ and CD23-, most of the experienced hematopathologists would agree that CD23- negative CLL/SLL are not uncommon. If a CD5+, CD23-negative small B-cell neoplasm is encountered, to determine if it is a CD23-negative CLL/SLL or MCL, other immunological markers will be

Table 3 Features differentiating CLL/SLL and MCL

Markers	CLL/SLL	MCL
Surface		
CD19	+	+
CD20	Low density+	High density+
CD22	Low density+	High density+
Ig (κ/λ)	Low density+	High density+
CD5	+	+
CD23	+	-
CD43	+	-
CD11c	Low density+	-
FMC7	-	+
CD79a	-	+
Nuclear		
Cyclin D1	-	-
Cytogenetics		
T(11;14)	-	+
Del13q14	+	-
Trisomy 12	+	-/+

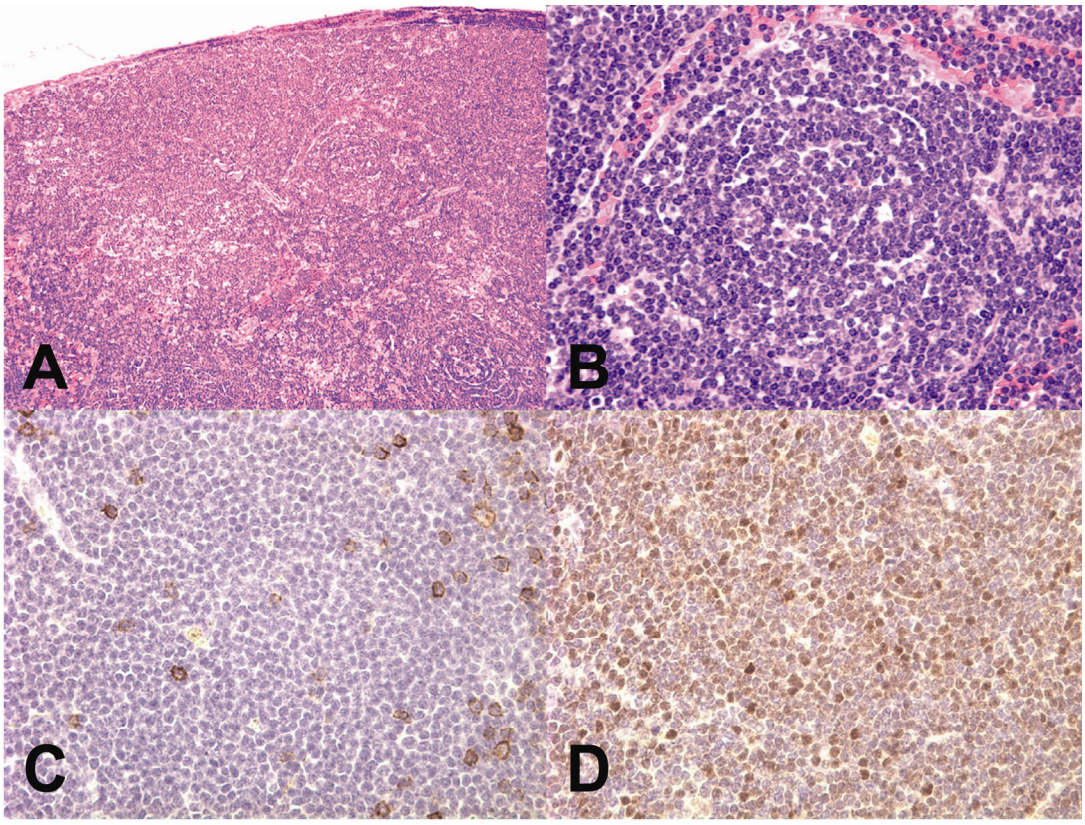

Figure 4 CD5-negative MCL under low power (**A**) and medium power (**B**). The lymphoma cells are negative for CD5 (**C**) but positive for cyclin D1 (**D**).

helpful (**Table 3**). Low density expression of CD20, CD22 and surface immunoglobulin and absent FMC7 expression favor the diagnosis of CLL/SLL. The opposite is true for MCL. Finally, t(11;14) and cyclin D1 would be most useful in differentiating these two entities. However, even cyclin D1 results can occasionally be misleading (see below).

Pitfall 2: CD5-negative MCL

We have encountered a 62-year-old man with lymphadenopathy. The biopsy showed a lymph node effaced by a vaguely nodular lymphoid proliferation composed of a monotonous population of small to medium-sized cells (**Figure 4A**). Some of the neoplastic cells arrange in a concentric pattern with no remnant germinal centers (**Figure 4B**). The neoplastic cells are CD20+, but negative for CD3, CD5 or CD10 by immunohistochemistry. Although the CD5 negativity may exclude either SLL/CLL or MCL, the cytological features are suggestive of MCL. Immunostain for cyclin D1 was performed and the lymphoma cells were strongly positive (**Figures 4C** and **4D**). In 2002, Liu *et al* identified 25 cases of CD5-negative MCL with cyclin D1 expression and t(11;14) [12]. In real-time practice, we do not know how many cases of CD5-negative MCL had been misdiagnosed as marginal zone B-cell lymphoma due to the negativity for CD5 and CD10. Fortunately, it was believed that these CD5-negative MCL might be more indolent. Some pathologists may argue that these lymphomas may be truly marginal zone B-cell lymphomas with aberrant cyclin D1 expression since they are more indolent than classical MCL. Since cyclin D1 plays an important role in MCL and the morphology is more consistent with MCL, for this case, a diagnosis of CD5-negative MCL was favored. Future molecular profiling may be helpful in resolving this issue. Indeed, in any cases of B-cell lymphoma of small cell type, I would always perform immunostain for cyclin D1 irrespective of CD5 expression. We cannot afford to miss a MCL since it is well accepted that MCL is more aggressive than other B-cell lymphomas of small cell type.

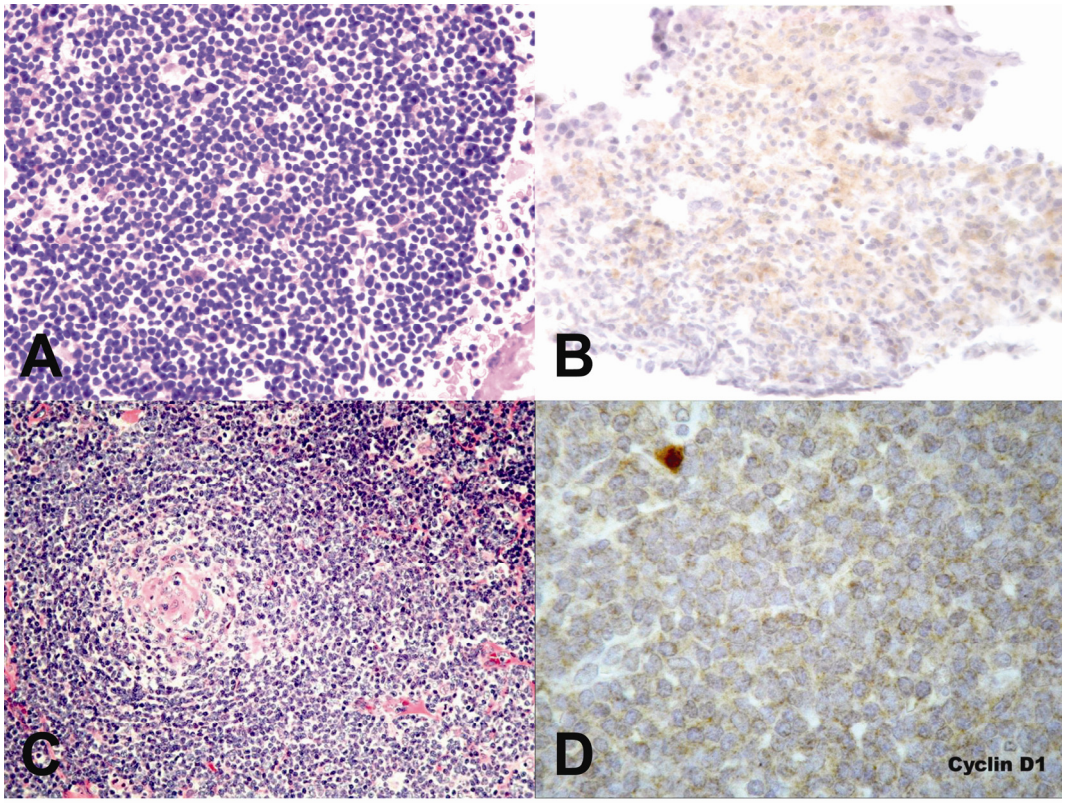

Figure 5 Cyclin D1-negative MCLs. Bone marrow involvement by a monotonous population of small to medium-sized lymphoid cells suggestive of MCL morphologically and immunophenotypically (**A**). However, immunostain for cyclin D1 was negative (**B**). Nodal MCL with a typical "mantle zone" pattern (**C**) but negative for cyclin D1 expression (**D**) (courtesy of Dr. Dennis Weisenburger).

Pitfall 3: Cyclin D1-negative MCL

If CD5 negativity cannot rule out MCL, can a negative cyclin D1 immunostain do? We had a bone marrow biopsy from a 57-year-old patient with both paratrabecular and interstitial infiltrations by a monotonous population of small to medium-sized lymphoid cells. The aspirate smear showed that these cells had centrocytic morphology (**Figure 5A**). Flow cytometry revealed that these cells expressed high density CD20 and surface immunoglobulin lambda light chain. These cells were also CD5+, but negative for CD10 and CD23. All these features were consistent with MCL. However, immunostain for cyclin D1 was negative (**Figure 5B**). Dennis Weisenburger reported a case of a 61-year-old man whose lymph node biopsy revealed a morphologically typical MCL (**Figure 5C**) but was negative for cyclin D1 (**Figure 5D**). However, using immunohistochemical staining with antibodies against cyclin D2 and D3 he found it to be positive for cyclin D3, and thus called it "cyclin D1-negative MCL" [13]. His group also studied 6 cases of cyclin D1-negative MCL using gene array analysis and found that these lymphoma cells had a similar genetic signature as the cyclin D1-positive MCL except for the lack of cyclin D1 gene expression [14]. Instead of cyclin D1, these cases expressed either cyclin D2 or D3. Their studies not only confirmed that MCL is a distinct biologic entity, but also showed that cyclin Ds, including cyclin D1, are the key molecules that lead to the development of MCL. It is possible that our case may represent a cyclin D1-negative MCL. Unfortunately, cyclin D2 and D3 stains are currently not widely used in most of the hematopathology laboratories.

Pitfall 4: CLL/SLL with Focal Cyclin D1 Staining

To make things even more complicated, one group recently reported cyclin D1 staining in a *bona fide* CLL/SLL [15]. However, cyclin D1

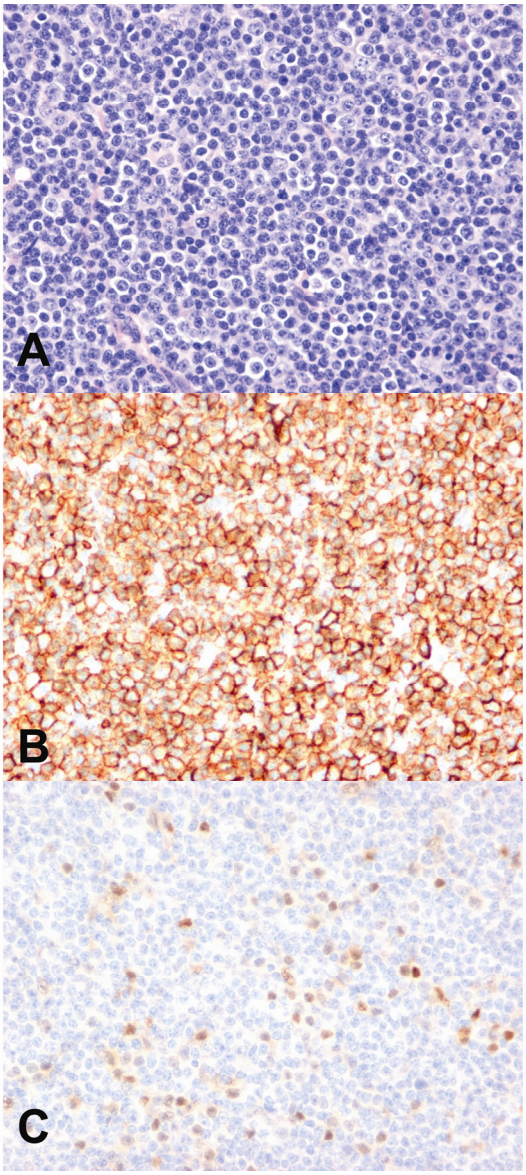

Figure 6 CLL/SLL with focally increased large paraimmunoblasts in the proliferation center (**A**) positive for CD20 (**B**) and focally positive for cyclin D1 (**C**, positive in approximately 10-15% of the lymphoma cells).

expression in that case was focal and only present on less than 10% of the neoplastic cells. We also encountered two such cases with focal cyclin D1 positivity (**Figure 6**), but no other evidence of MCL. Whether these cases represent an aggressive subset of CLL/SLL or a distinct entity is currently unknown. Larger series and more extensive immunological, cytogenetic and molecular studies are required to correlate with the clinical course and outcome of these cases.

Pitfall 5: T(11;14)-negative, Trisomy 12+ MCL

Although t(11;14) is characteristic of MCL [16], more and more data suggests that t(11;14)-negative MCL does exist. Some of the pseudo-negative cases may reflect the limitation of our current detection assays [17]; others are probably truly t(11;14)-negative MCL cases, such as cyclin D1-negative MCL [14]. Just as the presence of t(11;14) and cyclin D1 positivity excludes CLL/SLL, pathologists use the chromosomal abnormalities del13q14 and trisomy 12 to exclude MCL [15]. Trisomy 12 is considered the second most common cytogenetic abnormality in CLL/SLL, which is usually associated with frequent atypical morphology, increased prolymphocytes and an adverse prognosis [18]. However, trisomy 12 has been reported in MCLs [19]. A recent expression profile analysis of a trisomy 12+, t(11;14)-negative mature B-cell lymphoma showed a molecular signature consistent with MCL [20], suggesting overlaps between CLL/SLL and MCL. With various future molecular profiling approaches, the differentiation of CLL/SLL and MCL will be beyond the reach of morphology, immunology and even cytogenetics.

In summary, differentiating CLL/SLL from MCL is not as straight forward as we thought. We have to integrate all the information, including morphology, immunohistochemistry, flow cytometry, cytogenetics and molecular studies, to reach the final diagnosis. Translational research will guide us in search for more specific molecular markers. To differentiate CLL/SLL from MCL effectively, we need to perform cyclin D1 immunostain on all small B-cell lymphomas, and to correlate morphology with flow cytometric profile of the lymphoma cells. In difficult cases, for example, when MCL is suspected and cyclin D1 is negative, immunostains for cyclin D2 and D3 should be performed if available. Whenever possible, cytogenetics and fluorescence in situ hybridization should be performed to identify the characteristic chromosomal abnormalities associated with SLL/CLL and MCL.

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References

[1] Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA, Bloomfield CD and Lander ES. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. *Science* 1999;286: 531-537.

[2] Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J Jr, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO and Staudt LM. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;403: 503-511.

[3] Mitsiades CS, Mitsiades N, Treon SP and Anderson KC. Proteomic analyses in Waldenstrom's macroglobulinemia and other plasma cell dyscrasias. *Semin Oncol* 2003; 30:156-160.

[4] Glas AM, Kersten MJ, Delahaye LJ, Witteveen AT, Kibbelaar RE, Velds A, Wessels LF, Joosten P, Kerkhoven RM, Bernards R, van Krieken JH, Kluin PM, van't Veer LJ and de Jong D. Gene expression profiling in follicular lymphoma to assess clinical aggressiveness and to guide the choice of treatment. *Blood* 2005;105:301-307.

[5] Lamant L, de Reyniès A, Duplantier MM, Rickman DS, Sabourdy F, Giuriato S, Brugières L, Gaulard P, Espinos E and Delsol G. Gene-expression profiling of systemic anaplastic large-cell lymphoma reveals differences based on ALK status and two distinct morphologic ALK+ subtypes. *Blood* 2007;109:2156-2164.

[6] D'Arena G, Di Renzo N, Brugiatelli M, Vigliotti ML and Keating MJ. Biological and clinical heterogeneity of B-cell chronic lymphocytic leukemia. *Leuk Lymphoma* 2003;44:223-228.

[7] Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Lichtman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini M and Chiorazzi N. IgV gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood* 1999;94:1840-1847.

[8] Hamblin TJ, Davis Z, Gardiner A, Oscier DG and Stevenson FK. Unmutated immunoglobulin VH genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood* 1999;94:1848-1854.

[9] Hamblin TJ, Orchard JA, Ibbotson RE, Davis Z, Thomas PW, Stevenson FK and Oscier DG. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. *Blood* 2002;99:1023-1029.

[10] Calin GA, Ferracin M, Cimmino A, Di Leva G, Shimizu M, Wojcik SE, Iorio MV, Visone R, Sever NI, Fabbri M, Iuliano R, Palumbo T, Pichiorri F, Roldo C, Garzon R, Sevignani C, Rassenti L, Alder H, Volinia S, Liu CG, Kipps TJ, Negrini M and Croce CM. A MicroRNA signature associated with prognosis and progression in chronic lymphocytic leukemia. *N Engl J Med* 2005;353:1793-801.

[11] Bertoni F and Ponzoni M. The cellular origin of mantle cell lymphoma. *Int J Biochem Cell Biol* 2007;39:1747-1753.

[12] Liu Z, Dong HY, Gorczyca W, Tsang P, Cohen P, Stephenson CF, Berger CS, Wu CD and Weisberger J. CD5- mantle cell lymphoma. *Am J Clin Pathol* 2002;118:216-224.

[13] Weisenburger DD. Nodular Mantle Cell Lymphoma, Cyclin D1-, Cyclin D3+. *USCAP* 2004, Hematopathology Specialty Conference Case #3.

[14] Fu K, Weisenburger DD, Greiner TC, Dave S, Wright G, Rosenwald A, Chiorazzi M, Iqbal J, Gesk S, Siebert R, De Jong D, Jaffe ES, Wilson WH, Delabie J, Ott G, Dave BJ, Sanger WG, Smith LM, Rimsza L, Braziel RM, Müller-Hermelink HK, Campo E, Gascoyne RD, Staudt LM and Chan WC. Lymphoma/Leukemia Molecular Profiling Project: Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. *Blood* 2005;106:4315-4321.

[15] O'Malley DP, Vance GH and Orazi A. Chronic lymphocytic leukemia/small lymphocytic lymphoma with trisomy 12 and focal cyclin d1 expression: a potential diagnostic pitfall. *Arch Pathol Lab Med* 2005;129:92-95.

[16] Lukas J, Jadayel D, Bartkova J, Nacheva E, Dyer MJ, Strauss M and Bartek J. BCL-1/cyclin D1 oncoprotein oscillates and subverts the G1 phase control in B-cell neoplasms carrying the t(11;14) translocation. *Oncogene* 1994;9: 2159-2167.

[17] Evans PA, Pott Ch, Groenen PJ, Salles G, Davi F, Berger F, Garcia JF, van Krieken JH, Pals S, Kluin P, Schuuring E, Spaargaren M, Boone E, González D, Martinez B, Villuendas R, Gameiro P, Diss TC, Mills K, Morgan GJ, Carter GI, Milner BJ, Pearson D, Hummel M, Jung W, Ott M, Canioni D, Beldjord K, Bastard C, Delfau-Larue MH, van Dongen JJ, Molina TJ and Cabeçadas J. Significantly improved PCR-based clonality testing in B-cell malignancies by use of multiple immunoglobulin gene targets. Report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia* 2007;21:207-217.

[18] Matutes E, Oscier D, Garcia-Marco J, Ellis J, Copplestone A, Gillingham R, Hamblin T, Lens D, Swansbury GJ and Catovsky D. Trisomy 12 defines a group of CLL with atypical morphology: correlation between cytogenetic, clinical and laboratory features in 544 patients. *Br J Haematol* 1996;92:382-388.

[19] Matutes E, Carrara P, Coignet L, Brito-Babapulle V, Villamor N, Wotherspoon A and Catovsky D. FISH analysis for BCL-1 rearrangements and trisomy 12 helps the diagnosis of atypical B cell leukaemias. *Leukemia* 1999;13:1721-1726.

[20] Finn WG, Sreekumar A, Menon A, Utiger C and Chinnaiyan A. Trisomy 12-associated, t(11;14)-negative mature B-cell leukemia with gene expression profile resembling mantle cell lymphoma. *Leuk Lymphoma* 2006;47:121-127.