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XII. Gray zone lymphomas: a biological experiment, and a challenge for diagnosis and management

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introduction

Considerable progress has been made regarding the origin of the neoplastic cell in the lymphomas, both classical Hodgkin lymphoma (CHL), and the non-Hodgkin lymphomas (NHLs). As CHL is derived from an altered B lymphocyte, it is not surprising that areas of overlap with B-cell NHL should occur both biologically and clinically. The Hodgkin–Reed Sternberg (HRS) cell is a crippled B cell, incapable of immunoglobulin secretion, and many other aspects of the B-cell program are suppressed as well [1]. The biological and molecular events leading to this state are complex, and not fully resolved.These events may occur in the context of a relatively normal immune system as seen in most patients with CHL, or in the setting of immunodeficiency. The study of lymphomas at the interface of CHL and NHL may provide insight into the pathogenesis of *de novo* CHL, and the molecular and cellular events distinguishing it from NHL.

The term gray zone lymphoma (GZL) has been used generically to describe a process at the histological, and also biological, interface between various types of lymphoma [2]. It has been used in the context of CHL and NHL, and also nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) and B-cell lymphomas, most commonly T-cell/histiocyte-rich large B-cell lymphoma (TCHRLBCL). Similarly, synchronous and metachronous lymphomas of discordant histology can involve a combination of HL and NHL. We limit the use of the term gray zone lymphoma to those cases in which there are morphological, biological, and clinical features suggesting overlap between HL and NHL. A similar but unrelated diagnostic issue involves those cases in which there may be diagnostic uncertainty, but not true biological overlap, such as HRS-like cells encountered in other benign and malignant conditions. These lesions are best discussed in the context of differential diagnosis, and will not be covered here, as they do not represent a true biological borderland.

B-cell lymphoma, unclassifiable, with features intermediate between diffuse large B-cell lymphoma (DLBCL) and CHL was incorporated for the first time in the 4th edition of the World Health Organization (WHO) Classification of Tumours of Haematopoietic and Lymphoid Tissues in 2008 [3]. Despite its inclusion in the recent WHO classification, this type of lymphoma, also known more conveniently as GZL, still represents a major challenge to both the pathologist and the clinician.

GZL designates a variant of lymphoma combining features of CHL and DLBCL, especially primary mediastinal large B-cell lymphoma (PMBL), and was first described by Traverse-Glehen et al. in 2005 [4]. While these lymphomas most commonly present with mediastinal disease, similar cases have been described in peripheral lymph nodes without mediastinal involvement. Characteristically, GZLs demonstrate histologic and immunophenotypic features of both PMBL/DLBCL and CHL, precluding classification as PMBL/DLBCL or CHL. Most often, there is asynchrony between morphology and immunophenotype, which causes difficulties in establishing a definitive diagnosis. From a clinical perspective, GZLs represent an aggressive lymphoma subtype and the optimal therapy is as yet undetermined.

Instances of either synchronous or metachronous occurrences of CHL and PMBL are closely related biologically and conceptually, but from the standpoint of nomenclature, are not included in the WHO category as currently defined. Importantly, such cases with distinct histological and immunophenotypic patterns occurring in the same patient have been shown to be clonally related. Interestingly, when PMBL and the nodular sclerosis subtype of CHL (CHLNS) are seen at different points in time, both sequences are encountered, suggesting that the transformation from CHL to PMBL is a two-way street [4].

One ambiguity in the published WHO definition is the inclusion of cases positive for Epstein–Barr virus (EBV). Many EBV-positive B-cell proliferations may show immunophenotypic and histological overlap with CHL. For example, the EBV+ lymphoproliferations associated with methotrexate or other immunosuppressive agents may closely simulate CHL [5]. Similarly, EBV+ large B-cell lymphomas seen most often in the elderly, and associated with immune senescence, may resemble CHL both morphologically and immunophenotypically [6]. While the current WHO monograph definition states that up to 20% of cases of GZL can be EBV+, it is likely that this is an unrelated phenomenon to the more common mediastinal GZL, which are essentially always negative for EBV. It has become apparent that the immunophenotypic profile of CHL (CD30+, CD15+, CD20 weak/variable) is not specific to CHL, and can be encountered in particular in EBV-driven B-cell proliferations unrelated to CHL.

morphologic and immunophenotypic features of GZL

Morphologically, GZLs present with pleomorphic tumor cells that often sheet-out and grow in a diffusely fibrotic stroma. Tumor cells can be reminiscent of PMBL or CHL with a broad spectrum of cytological appearance in different areas of the tumor. The inflammatory infiltrate is usually sparse, but scattered eosinophils, lymphocytes and histiocytes may be present [3]. Immunophenotypically, the tumor cells present a pattern with transitional features between CHL and PMBL, particularly with asynchrony between morphology and immunophenotype. GZL can present with a Hodgkin-like morphology and a phenotypic pattern of PMBL (CD20++, CD15–) or the lymphoma can present with a PMBL-like morphology and a Hodgkin phenotype with expression of CD30, CD15 and loss of CD20 and CD79a. The transcription factors PAX5, OCT-2 and BOB.1 are positive in most cases. A recent study by Hoeller et al. [7] investigated further immunohistochemical markers, which might be of help for distinguishing between PMBL and CHL. They proposed a positive predictive value and high sensitivity of p63 for PMBL and cyclin E for CHL and suggested that an investigation of GZL with these markers might prove useful. However, in recent preliminary data, Summers et al. [8] found that both p63 and cyclin E were co-expressed in a high proportion of cases of GZL, and that expression of one or another marker did not appear to delineate meaningful subsets. The relatively high incidence of cyclin E positivity might suggest that GZLs are closer overall to CHL than to PMBL.

clinical features of GZL

PMBL and CHLNS share a number of common clinical characteristics. Both lymphomas frequently present in young women with an anterior mediastinal mass involving the thymus gland and supraclavicular lymph nodes. The occurrence of PMBL and CHLNS as composite lymphoma in the same anatomic site and sequential occurrences of PMBL and CHLNS in the same patient at different time points have also been reported [4]. Except for a male predominance, GZLs have a clinical presentation similar to that of PMBL and CHLNS, underscoring the close relationship between these three lymphomas [4]. However, this diagnostic gray zone is not only of theoretical interest, but more importantly represents a practical problem, as the treatment approaches for CHL traditionally differ from those for aggressive B-cell lymphomas. Clinical experience shows that GZLs fail to respond to therapeutic regimens effective in the parent entities CHL or PMBL. In a first clinical trial of this rare entity, 11 patients with GZL and 35 patients with PMBL were treated with a DA-EPOCH-R regimen at the National Cancer Institute [9]. Confirming prior reports outside the setting of a clinical trial, preliminary data indicate that GZLs generally have a more aggressive clinical course than PMBL or CHL, and that patients in this study required radiation therapy for sustained failure-free survival [9]. Therefore, the exploration of the biological basis for the poor clinical responsiveness of this tumor is an essential step in order to develop new and more effective therapies for GZL. Recent studies provide further insights to the mechanisms involved in modulating the plasticity of the neoplastic B cell and tumor microenvironment in CHL and PMBL [10]. Moreover, having new biological tools to diagnose GZL could help to accurately identify those patients requiring distinctive management.

biological features of GZL

From a biological perspective, gene expression profiling studies revealed that PMBL and CHLNS share a common gene expression signature [11–12]. Additionally, a number of common genetic aberrations in PMBL and CHL further underscore their close relationship [13]. PMBLs show frequent gains of gene regions on chromosomes 9p and 2p that were also described in CHL but only rarely detected in DLBCL. A recent study of chromosomal aberrations for 2p16.1, 9p24.1 and 8q24 in children with PMBL found similar frequencies of investigated loci as compared with adults [14]. Of note, one case of pediatric GZL was also accessible for genetic studies, but did not reveal any alterations.

The exact molecular mechanisms responsible for the transformation of a B cell to a HRS cell are not fully understood. Recent studies have suggested that downregulation of the B-cell program in CHL may be responsible for tumorigenesis and these modifications may be controlled at the epigenetic level [15–16]. GZLs, mediastinal composite lymphomas and mediastinal sequential lymphomas represent a unique resource to study this question, since the cells appear to have the capacity to undergo reprogramming of their phenotype during the disease course. A recent large-scale methylation analysis showed a close epigenetic relationship between GZL, CHLNS and PMBL, but remarkably different from that of DLBCL. Moreover, principle component analysis indicated that GZL did not cluster with either CHL or PMBL, but demonstrated a unique epigenetic signature, validating its inclusion in the WHO classification as a separate disease [10]. Importantly, GZL could be distinguished from CHLNS and PMBL by differential methylation of selected CpG islands, and a class prediction model could be established to segregate the various entities. Thus, the epigenetic signature of these lymphoma entities may not only be useful in establishing new diagnostic tools and in clarifying the pathogenesis of these lymphomas, but also may help to identify possible targets for future therapies. Although material is limited due to the rarity of GZL, further studies are urgently needed to explore this entity in more detail. Gene expression profiling and genetic studies such as FISH might be of great value for a more comprehensive understanding of disease pathogenesis and establishing new and more effective therapies for those patients.

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