non-Hodgkin lymphomas. However, Brune and colleagues demonstrated through gene expression profiling analysis that NLPHL most closely overlaps with T-cell–rich B-cell lymphoma (TCR-BCL), a subset of DLBCL, and cHL.[3] The histologic variants of NLPHL as described by Fan et al were found to be associated with both higher rates of relapse and advanced stages of disease.[4] In addition, the presence vs absence of these histologic variants forms a key component of the prognostic scoring model developed by the German Hodgkin Study Group (GHSG). In the GHSG model, 3 distinct risk factor groups were based on variant histopathologic growth pattern, low serum albumin, and male sex, which are incorporated into treatment selection criteria, moving beyond use of just stage alone.[5]

Prior to the current reports, one of the largest retrospective series was published by the British Columbia Cancer Agency (BCCA). Fourteen percent of 95 NLPHL patients followed experienced transformation to an aggressive BCL at a median of 8.1 years, with an actuarial risk of transformation at 20 years of 30%.[6] The BCCA database review describes not only a higher rate of transformation but also a delayed time frame for these transformations by an increase of about 5 years when compared with the median time to transformation in the Kenderian et al article of 2.9 years. However, similar to the Mayo Clinic, the BCCA also described splenic involvement as a risk factor for transformation. Kenderian et al also described that past treatment with chemotherapy also increased risk of transformation potentially because patients believed to have higher risk disease were treated with chemotherapy.

Given the rarity of the diagnosis, the challenge that still remains is what is the optimal treatment of a patient who has a higher risk for disease transformation based on clinical or biologic features. In both the Mayo and BCCA articles, the majority of the patients who received chemotherapy for NLPHL before transformation were treated with ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) or ABVD-like regimens, and then at transformation many were treated with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) ± R (rituximab). Data from both centers thus raises the question of alternative regimens for patients with the highest risks for transformation. In support of this, the Princess Margaret group has described, for advanced-stage patients, a high relapse rate of over 50% at 5 years for NLPHL patients when treated with ABVD including 4 patients who developed transformation.[7] Taken together, this suggests that, for patients identified as having high risk of transformation, alternative regimens beyond ABVD potentially be considered. The challenge though is identifying what that regimen should be. We at the MD Anderson Cancer Center have used a regimen based on R-CHOP and have not seen transformations. But only through large cooperative clinical trials can we determine whether R-CHOP or other more novel regimens are actually superior to ABVD or R-ABVD for patients at high risk of transformation.[8]

In conclusion, these data from Kenderian et al provide reassurances to clinicians who care for patients with NLPHL in that (1) even if transformation occurs it is generally at a low rate and (2) with additional treatment these patients do well with survival equal to their nontransformed NLPHL counterparts. Prospective multicenter clinical therapeutic trials, however, remain vital to continue to advance how we care for these patients with this very rare hematologic diagnosis.

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● ● ● LYMPHOID NEOPLASIA

Comment on Kanakry et al, page 2007

When kissing (disease) counts

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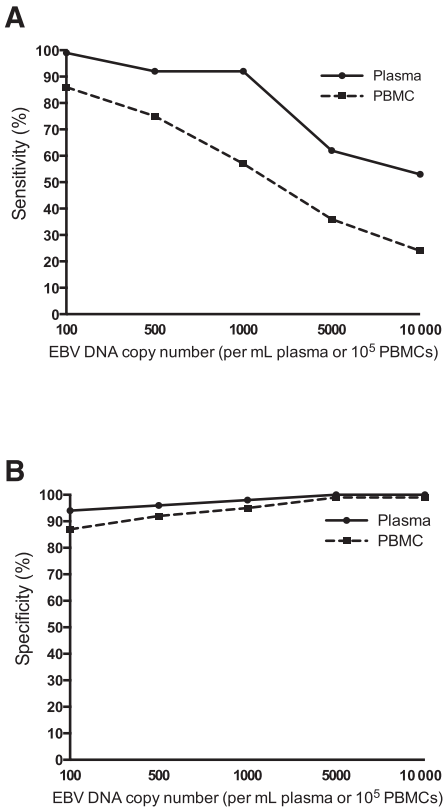
In this issue of *Blood*, Kanakry et al analyzed Epstein-Barr virus (EBV) DNA expression in peripheral blood cellular compartments from 2146 patients with various malignancies and found that cell-free plasma EBV DNA was a better marker of EBV$^+$ disease than EBV DNA from peripheral blood mononuclear cells (PBMCs).[1]

EBV is a human herpesvirus which primarily infects B cells and epithelial cells. It is one of the most common viruses, with >90% of adults having evidence of previous infection. In children, the symptoms (if any) are usually mild and flulike, whereas in adolescents and young adults, EBV can cause infectious mononucleosis (IM), also known as glandular fever or "kissing disease." Once infected with EBV, the virus remains latently present in B cells in the blood for the rest of the individual's life. In addition, EBV infection has been associated with the development of a variety of human malignancies, including several types of lymphoma, lymphoproliferative disorder, and nasopharyngeal carcinoma (NPC).[2]

In the present study, plasma and PBMC samples of 2146 patients with various diseases, including IM, hemophagocytic lymphohistiocytosis, posttransplantation



EBV DNA in plasma had better sensitivity (A) and specificity (B) than PBMCs in distinguishing between patients with active, systemic EBV$^+$ disease and patients that did not have prior, current, or subsequent history of EBV$^+$ disease. See Figure 2B-C in the article by Kanakry et al that begins on page 2007.

lymphoproliferative disorder, non-Hodgkin lymphoma, Hodgkin lymphoma, lymphoproliferative disorder, NPC, and oral hairy leukoplakia, are analyzed for EBV DNA expression and its association with EBV$^+$ disease. The authors show that EBV DNA in plasma has a higher sensitivity and specificity for distinguishing patients with an active, systemic EBV$^+$ disease from patients with no prior, current, or subsequent history of EBV$^+$ disease than PBMCs (see figure). They describe a positive correlation between EBV DNA copy number and active, systemic EBV$^+$ disease, compared with patients with EBV$^+$ disease in remission and those with no EBV$^+$ disease, both in PBMCs and plasma. Finally, in patients with no EBV$^+$ disease, EBV DNA is more often present in PBMCs than in plasma. These data lead to the conclusion that cell-free (plasma) EBV DNA performs better than cellular (PBMC) EBV DNA as a marker for EBV$^+$ disease.

The clinical importance of this study lies in the fact that it was done on a very large set of patients, and that it helps to bring clarity to the conflicting data that have been published so far on which blood component is best to be used for EBV DNA expression as a marker for EBV$^+$ disease.[3,4] Several additional studies on smaller sets of patients were recently published, reporting that EBV DNA positivity was associated with worse survival in chronic lymphocytic leukemia (CLL).[5,6] All these together support the concept that development of vaccines against EBV in patients at risk for aggressive disease could be a future prophylactic approach for the prevention of the deadly metastatic disease or of Richter transformation. Furthermore, EBV-derived biomarkers can be used to predict the natural history of an EBV$^+$ cancer.

Although Kanakry et al found that detection of EBV DNA in plasma was uncommon in the absence of EBV$^+$ disease, there were still 6% of patients in which EBV DNA was detected in plasma although they did not present with EBV$^+$ disease. Therefore, it might be worth considering markers other than EBV DNA to screen for EBV-associated diseases or for evaluating response to therapy. In this regard, Epstein-Barr viral microRNAs (miRNAs) may be of use. EBV miRNAs are located within 2 transcripts of the viral genome, the *Bam*HI fragment H rightward open reading frame 1 (BHRF1) and the *Bam*HI A rightward transcripts (BARTs).[7] Recently, the involvement of EBV viral miRNAs in some EBV-associated malignancies was described. For example, viral miRNA BHRF1-1 is expressed significantly higher in plasma of patients with CLL when compared with normal individuals, and BHRF1-1 expression in B cells of patients with CLL is associated with shorter overall survival.[8] Similarly, Zhang et al[9] found expression of BART7 and BART13 viral miRNAs in plasma of patients with NPC, whereas these miRNAs are only rarely expressed in healthy individuals and non-NPC tumor patient controls. In addition, higher EBV miRNA expression is associated with advanced disease stages, and the expression is reduced upon radiotherapy.[9] Also in NPC, BART17 was found to be highly expressed in plasma samples from NPC patients, when compared with non-NPC controls.[10]

Whether expression of EBV miRNAs can be used as a biomarker for other EBV- associated diseases still remains to be determined, but the first reports seem promising and the research worth continuing.

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