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# The Utility of Phosphohistone-H3 (PHH3) Immunohistochemistry as an Adjunct in Grading Follicular Lymphoma

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## Background

Routine histologic grading of follicular lymphoma (FL) is currently performed by examining hematoxylin & eosin (H&E) stained sections and estimating the average number of centroblasts per 40x high power field (HPF) over ten representative neoplastic follicles.<sup>1</sup> Whether this is truly the best method for grading FL remains controversial.<sup>2,3,4</sup> The 2008 World Health Organization (WHO) classification endorsed the use of Ki67 immunohistochemistry (IHC) as a clinically justified adjunct due to general correlation of proliferation index with histologic grade<sup>1</sup>; however, interpretation of Ki67 has high interobserver variability<sup>5,6</sup>. In this study, the correlation between histologic grade and mitotic count (MC) was investigated using the mitosis-specific immunohistochemical stain phosphohistone-H3 (PHH3). Interobserver variability in H&E, Ki67 and PHH3 interpretation was also examined.

## Design

Follicular lymphoma cases available at Brooke Army Medical Center between January 2005 and April 2017 were identified. Cases were blinded, randomized, and reviewed by three staff hematopathologists. The average Ki67 proliferation index, MC per HPF using PHH3, MC per HPF on H&E stain, and number of centroblasts per HPF were recorded. Results were assessed for correlations and interobserver variability.

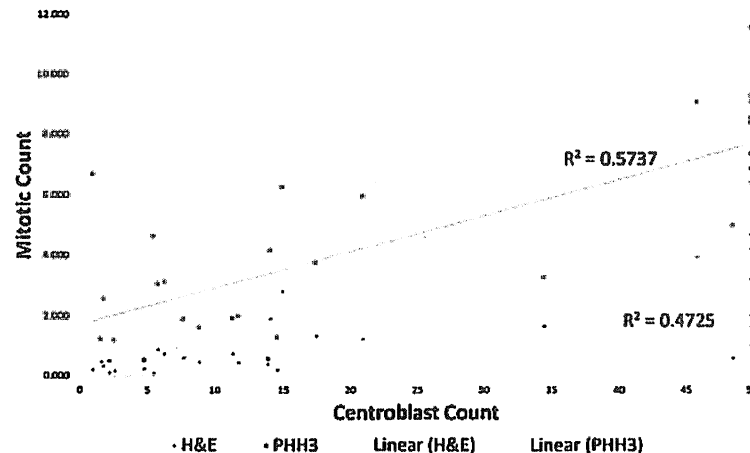


Figure 1. Mitotic count on H&E and PHH3 compared to centroblast count (Counts greater than 50 were recorded as 50)

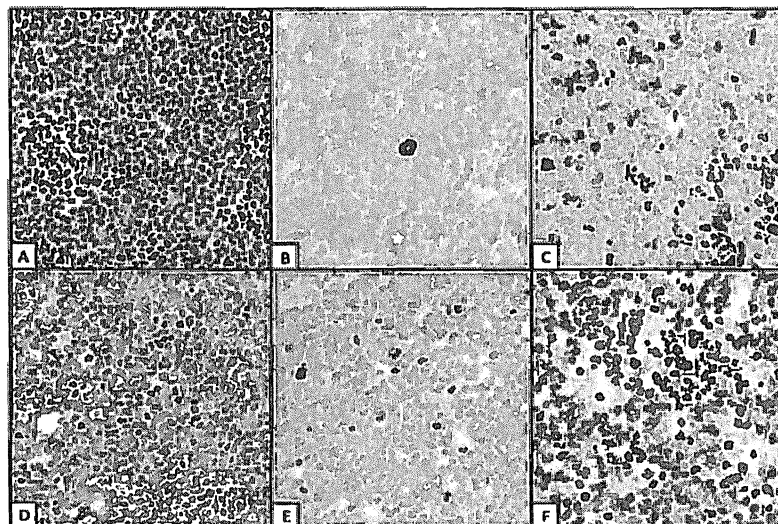


Figure 2. A-C) Low-grade (Grade 1) follicular lymphoma. 40x (H&E, PHH3, Ki67). D-F) High-grade (grade 3B) follicular lymphoma. 40x (H&E, PHH3, Ki67).

## Results

Thirty cases of FL were studied, including 17 low-grade (LGFL: 5 grade-1 and 12 grade-2) and 13 high-grade (HGFL: 12 grade-3A and 1 grade-3B). PHH3 MC resulted in the strongest correlation to grade ( $r = 0.773$ ) compared to Ki67 ( $r = 0.679$ ) and H&E MC ( $r = 0.562$ ) and the strongest linear relationship to centroblast count ( $p = 0.006$ ,  $R^2 = 0.574$ ) (Figure 1). The mean PHH3 MC for LGFL was 2.4 per HPF compared to a mean H&E MC of 0.7 per HPF (mean difference = 1.7,  $p < 0.0001$ , 95% CI [1.5 – 1.9]). The mean PHH3 MC for HGFL was 7.3 per HPF compared to a mean H&E MC of 2.4 per HPF (mean difference = 4.9,  $p < 0.0001$ , 95% CI [4.3 – 5.3]). Agreement amongst pathologists was strongest for PHH3 (intraclass correlation coefficient [ICC] = 0.89, 95% CI [0.81-0.94]) followed by Ki67 (ICC = 0.85, 95% CI [0.75-0.92]) and H&E MC (ICC = 0.79, 95% CI [0.65-0.88]). Examples of staining patterns demonstrated in Figure 2.

## Conclusions

PHH3 MC more strongly correlated with routine histologic grade of FL than Ki67 proliferation index or H&E MC and showed the greatest interobserver agreement. These results support the feasibility of using PHH3 as an adjunct in FL grading.

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