Although many lymphomas can be reliably classified according to the World Health Organization Classification of 2008, the differentiation between nodal marginal zone lymphoma (NMZL) and follicular lymphoma (FL) is problematic in some cases. In fact, NMZL is often diagnosed by exclusion, resulting in heterogeneity in the diagnostic category of NMZL. New markers for NMZL have been described, but they have not yet been tested in combination.

In this study, we compared multiple immunohistochemical markers for their use in distinguishing NMZL from FL. From the results obtained, we constructed an algorithm that combines these markers to help distinguish between FL and NMZL. Notably, this algorithm also contains a category of “B-cell lymphoma, unclassifiable”, thus underlining the difficulty that remains in distinguishing NMZL from FL.

For the initial test series, we selected 47 patients with FL with a chromosomal rearrangement of BCL2, and 44 patients with a diagnosis of NMZL or probable NMZL, from the archive of the Department of Pathology at the Radboud university medical center (Nijmegen, the Netherlands). For all NMZLs, BCL2 translocations were excluded using fluorescent in-situ hybridization with split-signal probes. Patient characteristics are described in Online Supplementary Table S1.

Expression of germinal center markers was not considered an exclusion criterion for a diagnosis of NMZL in this study.

As expected, immunohistochemistry showed significant differences between NMZL and FL (Table 1).

Overall, FLs were positive for germinal center markers (CD10, BCL6, LMO2, HGAL) and negative for MNDA and IRTA1 (Online Supplementary Figure S1). NMZLs mostly showed an opposite pattern with positivity for MNDA in approximately two thirds of cases, IRTA1 staining in approximately one fifth of cases and usually no staining with germinal center markers. However, all germinal center markers were positive in a subset of NMZLs, and similarly, FLs with expression of MNDA were also identified (Online Supplementary Figure S2).

Based on the immunohistochemistry results, a combination of markers were used to design an algorithm that helped to distinguish NMZL from FL (Figure 1). This algorithm was built empirically, allowing inclusion of a category of “B-cell lymphoma, unclassifiable” to prevent contamination of the NMZL category. As expected, this algo-

**Table 1. Immunohistochemistry results.**

<table>
<thead>
<tr>
<th>Number of positive cases n (%)</th>
<th>Sensitivity and Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial series</strong></td>
<td><strong>Validation series</strong></td>
</tr>
<tr>
<td><strong>NMZL</strong></td>
<td><strong>FL</strong></td>
</tr>
<tr>
<td>BCL6</td>
<td>5 (11)</td>
</tr>
<tr>
<td>CD10</td>
<td>8 (18)</td>
</tr>
<tr>
<td>HGAL</td>
<td>11 (25)</td>
</tr>
<tr>
<td>LMO2</td>
<td>12 (27)</td>
</tr>
<tr>
<td>4/4 GCM</td>
<td>2 (5)</td>
</tr>
<tr>
<td>MNDA</td>
<td>31 (70)</td>
</tr>
<tr>
<td>IRTA1</td>
<td>6 (14)</td>
</tr>
</tbody>
</table>

Figure 1. Immunohistochemical algorithm for separation of nodal marginal zone lymphoma (NMZL) from follicular lymphoma (FL). The algorithm starts at the top with a lymphoma that is considered to be either FL or NMZL. If all four germinal center markers (BCL6, CD10, LMO2, HGAL) are positive, a diagnosis of FL is made. If not, IRTA1 expression is determined. If IRTA1 is positive, a diagnosis of NMZL is made. If IRTA1 is negative, MNDA and germinal center markers are used to divide the remaining cases into three categories: NMZL for MNDA positive cases with positivity for none or only one germinal center marker, FL for MNDA negative cases with expression of 2 or 3 germinal center markers and low-grade B-cell lymphoma, unclassifiable for cases that do not fit into either of these two categories.

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</tbody>
</table>

N. G. C. M. 4 out of 4 germinal center markers positive; FL: follicular lymphoma; NMZL: nodal marginal zone lymphoma; Se: sensitivity; Sp: specificity.
rithm classified most lymphomas according to their original
diagnosis (Table 2). However, in the initial test series,
one case of FL was classified as NMZL, and 6 cases of
NMZL were classified as FL by the algorithm; a signifi-
cant proportion of cases (13%) were considered “B-cell
lymphoma, unclassifiable” by the algorithm. Most (75%)
of these unclassifiable cases had an original diagnosis of
NMZL.

To validate the algorithm, a second validation group of
21 FLs and 13 NMZLs, collected from the archive of the
Department of Pathology at the Hospital del Mar
(Barcelona, Spain) was stained for the same markers as
the initial group. Overall, staining results were compara-
bly to those in the test group, with a high sensitivity of
BCL6 for FL and a high specificity of IRTA1 for NMZL
(Table 1); CD10 expression had a higher sensitivity in
comparison to the test group, but a lower specificity.
LMO2 and HGAL were less sensitive but more specific.

In this validation group, the algorithm gave a concur-
dant classification as either NMZL or FL in 85% of cases
(Table 2). No follicular lymphoma was misclassified as
NMZL, and only one NMZL was misclassified as FL. Four
cases (12%) were considered unclassifiable, three of
which had an original diagnosis of FL and one with an
original diagnosis of NMZL.

The algorithm was designed based on a comparison of
NMZLs with FLs with a translocation involving BCL2. How-
ever because a BCL2 translocation can be demonstrat-
ated relatively easily, the actual problem we are faced
with in daily practice is the separation of NMZL from FL
without a BCL2 translocation. FLs with and without a
BCL2 translocation might be different from each other,
has been suggested by one gene expression study, and
also by a recent comparative genomic hybridization
study. In the latter study, genetic aberrations in FLs with-
out a BCL2 translocation bore more resemblance to those
in NMZL than those in FLs with a BCL2 translocation.
To address this problem, we tested a small series of 6 FLs
without a BCL2 translocation, which were all classified as
FL by the algorithm. This supports the idea that this algo-

Table 2. Algorithm results.

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Initial series</th>
<th>Validation series</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>FL</td>
</tr>
<tr>
<td>FL</td>
<td>43 (91)</td>
<td>6 (14)</td>
</tr>
<tr>
<td>B-cell lymphoma, unclassifiable</td>
<td>3 (6)</td>
<td>9 (20)</td>
</tr>
</tbody>
</table>

FL: follicular lymphoma; NMZL: nodal marginal zone lymphoma.
Acknowledgments: the authors would like to thank dr. Roncador for providing the MNDA antibody.

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Key words: nodal marginal zone lymphoma; follicular lymphoma; immunohistochemistry.

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