Use of Bayes Rule and MIB-1 Proliferation Index to Discriminate Spitz Nevus From Malignant Melanoma

Robin T. Vollmer, MD, MS

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Abstract

Differentiating Spitz nevus from malignant melanoma is difficult and controversial. Despite helpful lists of differential diagnostic features, uncertainty about the diagnosis often provokes some to stain the tumor for MIB-1 antibody to Ki-67 and measure the proliferation index (PI) of the tumor. Of the many reports about MIB-1 PI in Spitz nevi and melanoma, none have consolidated the information to provide guidelines for the predictive probability that a lesion is a Spitz nevus, given that the MIB-1 PI falls into a certain interval. The present study used previously published data and exponential and $\gamma$ probability density functions to model statistical distributions of PI, respectively, in Spitz nevi and melanomas and Bayes rule to estimate the predictive probability that a lesion is a Spitz nevus, given an observed PI. Results indicate that PIs more than 10% favor a melanoma diagnosis and PIs less than 2%, Spitz nevus. PI values between 2% and 10% yield various predictive values for Spitz nevus, depending on the a priori probability that the lesion is a Spitz nevus. The algorithm tabulates guidelines for the predictive probabilities of Spitz nevus given an observed PI.

Although the diagnosis of most nevi and melanomas is straightforward, when it comes to evaluating Spitz nevus, its variants, and a few other borderline melanocytic tumors, the differential diagnosis can be vexing. Whereas some have suggested that most Spitz nevi can be diagnosed with certainty regardless of patient age, others have emphasized overlapping histologic features or even overlapping biology between Spitz nevus and melanoma, together with a need for intermediate categories such as atypical Spitz tumors and spitzoid melanomas. Consequently, some have recommended complete excisions even when the diagnosis of Spitz nevus is made with certainty, and others have recommended sentinel node lymph node biopsy when the diagnosis is uncertain.

To address this issue not long ago, Piepkorn called attention to the probabilistic nature of the problem of differentiating Spitz nevus from melanoma and of predicting outcomes. He observed that few of the histologic criteria for Spitz nevus have been evaluated to the level of knowing Bayesian-derived predictive probabilities, and he used 10 cases of Spitz nevus to illustrate the relatively low sensitivity of many histologic criteria thought to be defining for Spitz nevus. Walsh et al emphasized the probabilistic nature of this differential diagnosis.

Coincident with these discussions about the differential diagnosis between Spitz nevus and malignant melanoma, some have reported proliferation indices (PIs) as measured by the MIB-1 antibody to Ki-67 in Spitz nevi and melanomas. In general they found that PIs in Spitz nevi were significantly lower than those in melanomas. Yet it has been unclear just how one can use a PI to discriminate between Spitz nevus and melanoma. For example, there are no general guidelines, but
instead individual results from separate studies, each of which comprises limited numbers of patients.

Several hurdles exist to the formation of guidelines for the PI in the differential diagnosis of Spitz nevus and melanoma. First, PI comprises a continuous phenomenon observed in nevi and in melanomas, so that before one can interpret a PI in any particular lesion, it is necessary to know the probability distribution functions for PIs in Spitz nevi and melanomas. In addition, few single medical centers have sufficient data to learn these distribution functions, because we do not routinely stain melanomas for Ki-67 and most of us have not stained many Spitz nevi for Ki-67.

This article tackles these issues by consolidating previously published data and by using 2 distribution functions to model PIs in Spitz nevi and in melanoma. Bayes rule is used to derive an algorithm for estimating predictive probabilities of Spitz nevus based on PI. This approach produces guidelines for using MIB-1–based PI to discriminate Spitz nevus from malignant melanoma.

Materials and Methods

Bayes Rule

What we require is a formula for the probability that a lesion is a Spitz nevus vs a malignant melanoma, given that the Ki-67 PI falls into a certain interval, symbolized as Ipi. Throughout this article, PI is defined as the percentage of cell nuclei staining for an antibody to Ki-67, and for the key results, I rely solely on data using MIB-1. Ipi is defined so that the PI is greater than some number a and less than or equal to a larger number b, that is:

**Equation 1**

\[ I_{pi} = \{ a < PI \leq b \} \]

By using probability notation, a formula is sought for the conditional probability P(Spitz | Ipi), with Ipi symbolizing the PI interval. Bayes rule provides such a formula and is written as:

**Equation 2**

\[
P(\text{Spitz} | \ I_{pi}) = \frac{P(\ I_{pi} | \ Spitz) \times P(\text{Spitz})}{P(\ I_{pi} | \ Spitz) \times P(\text{Spitz}) + P(\ I_{pi} | \ Mel) \times (1 - P(\text{Spitz}))}
\]

Bayes rule says that we can calculate the desired probability P(Spitz | Ipi) if we know the values of the 3 probabilities appearing on the right side of Equation 2: (1) P(Ipi | Spitz), the probability of PI in the interval Ipi given that the patient has a Spitz nevus; (2) P(Ipi | Mel), the probability of PI in the interval Ipi given that the patient has a melanoma; and (3) P(Spitz), the a priori probability that the lesion is a Spitz nevus vs melanoma.

There are 2 steps to choosing appropriate functions for P(Ipi | Spitz) and P(Ipi | Mel). The first is to select an appropriate form of probability density function, f(x), and for this step we need to examine the frequency distributions of data reported on individual patients or as arranged in relatively small subsets over small ranges of PIs, because the frequency distribution provides an estimate of f(x). For example, the vertical bars in Figure 1A show a consolidated frequency distribution of PI from 3 studies of Spitz nevi comprising a total of 72 patients. The second step involves choosing appropriate parameters for f(x), and for this step we can use data or previous studies that report just means. Thus, in what follows, I use previous studies providing detailed results to first examine the consolidated histograms and choose a form for f(x). Then I use the means in a larger set of studies to choose values of the parameters for f(x).

Use of Distribution Functions for P(Ipi)

The vertical bars in Figure 1B show an estimate of the distribution function of PI from the same 3 studies used for Figure 1A. The distribution function was estimated from the cumulative sum of patient numbers for each PI and then divided by the total. (The smooth lines in the 2 figures are discussed in the next section.) The distribution function often is symbolized as F(x), and for a continuous variable like PI, f(x) and F(x) are related to one another through calculus in Equation 3.

**Equation 3**

\[ F(a) = \int_{-\infty}^{a} f(x) \, dx \]

with the integration limits between 0 and a, because negative PI is not defined. Thus, frequency distributions of PI and distribution functions of PI are directly related to one another, so that one can be used to estimate the other.

The probability that PI is less than some value, a, is defined as equal to F(a). In other words,

**Equation 4**

\[ P(PI \leq a) = F(a) \]

The probability of observing a PI in the interval \( a < PI \leq b \), therefore, can be obtained from the distribution function of PI as follows:

**Equation 5**

\[ P(a < PI \leq b) = F(b) - F(a) \]

Equation 5 provides the method used to estimate the probability of PI occurring in some interval, Ipi. The values for F were obtained from the medical literature as follows.

Modeling the Distribution Function of Proliferation Rate in Spitz Nevi

The smooth line in Figure 1A comes from the exponential probability density function f(x), and the smooth line in Figure 1B comes from the corresponding exponential distribution function F(x). Thus, because the shapes of these lines follow the observed histograms of raw data for PIs in Spitz nevi, we can use the means of the frequency distributions for a and b to estimate the parameters for f(x).
nevi, I chose the exponential probability density function to model PI in Spitz nevi. In mathematical terms this choice implies that:

**Equation 6**

\[ F(x) = 1 - \exp(-\alpha \times x) \]

with \( \alpha \) the parameter of the exponential density function. Substituting this choice in Equation 5 implies that the probability of PI occurring in the interval \( Ipi \) for Spitz nevi is given by:

**Equation 7**

\[ P(a < PI \leq b | \text{Spitz}) = \exp(-\alpha \times a) - \exp(-\alpha \times b) \]

### Modeling the Distribution Function of Proliferation Rate in Melanoma

**Figure 2** demonstrates the consolidated frequency distribution of PIs in grouped results over narrow ranges of PI in 386 previously reported cases of melanoma.\(^{12,16,18,21,25,27}\) Because the shape of this frequency distribution matches that of a \( \gamma \) probability density function (smooth line in Figure 2), I chose the \( \gamma \) probability density function to model \( P(Ipi \mid \text{Mel}) \) for melanoma. The particular form of the \( \gamma \) probability density used in Figure 2 and that I subsequently used for melanoma is:

**Equation 8**

\[ f(x) = \beta^2 \times x \times \exp(-\beta \times x) \]

and \( \beta \) is the parameter for the function. The distribution function for this density is:

**Equation 9**

\[ F(a) = \int \beta^2 \times x \times \exp(-\beta \times x) \, dx \]

where the integration limits are from 0 to \( a \). The solution of this integral is found by integrating by parts and yields:
Weighted mean for melanoma with proliferation rates in otherwise unselected cases of Spitz nevi or melanoma, and studies that counted MIB-1+ nuclei uniformly in the cells of the dermis rather than at hot spots.

For each study, I estimated \( \alpha \) for Spitz nevi or \( \beta \) for melanomas from the reported mean values as follows: For example, the mean value of PI in the first study of Spitz nevi in Table 1 was 15.00. Thus, the maximum likelihood estimate of \( \alpha \) is \( 1/\text{mean} \) for Spitz nevi (Appendix 1). Similarly, the maximum likelihood estimate of \( \beta \) is \( 2/\text{mean} \) for melanoma (Appendix 1). Thus, I searched the literature to collect reported means of proliferation rates in Spitz nevi and melanomas. However, unlike choices of data used to examine the frequency distributions of PIs in Figures 1 and 2, I restricted my interest to studies that used only the MIB-1 antibody for proliferation, studies that reported proliferation rates in otherwise unselected cases of Spitz nevi or melanoma, and studies that counted MIB-1+ nuclei uniformly in the cells of the dermis rather than at hot spots.

For each study, I estimated \( \alpha \) for Spitz nevi or \( \beta \) for melanomas from the reported mean values as follows: For example, the mean value of PI in the first study of Spitz nevi in Appendix 1 was 4.00. Thus, the maximum likelihood estimate of \( \alpha \) for this study was 1/4.00 or 0.250. Similarly, the mean value of PI in the first study of melanomas in Table 1 was 15.00. Thus, the maximum likelihood estimate of \( \beta \) for this study was 2/15.00 or 0.133. Finally, I obtained overall weighted means of \( \alpha \) and \( \beta \) by weighting by the number of patients used in each study. The weighted mean for \( \alpha \) was 0.646, and the weighted mean for \( \beta \) was 0.140. Figure 3 shows the exponential probability density function for Spitz nevi with \( \alpha \) equal to 0.646 (upper curve at left) and the \( \gamma \) probability density function for melanomas with \( \beta \) equal to 0.140 (flatter curve with broad peak).

**Choices for the A Priori Probability of Spitz Nevus, P(Spitz)**

The aforementioned developments leave one unknown remaining before Bayes rule (Equation 2) can be applied, namely, P(Spitz), the a priori probability that the lesion is a Spitz nevus before proliferation is considered. P(Spitz) provides a probabilistic estimate of the likelihood of a lesion being a Spitz nevus based on clinical and histologic characteristics other than proliferation rate. The choice of P(Spitz) should reflect clinical and histologic observations. For example, P(Spitz) can incorporate Bayes rule and distributions of age in Spitz nevi and melanoma. In addition P(Spitz) should reflect our subjective estimate of the probability of Spitz nevus based on routine histologic findings. For example, a logical approach might be to first base P(Spitz) on consideration of age alone and then alter this up or down depending on the histologic features of the lesion.

To illustrate the use of Bayes rule for PI, I chose 4 values for P(Spitz). For the situation when the data before PI favored Spitz nevus strongly, I assigned P(Spitz) to be 0.9. For the situation when the previous data favored melanoma, the a priori probability that the lesion is a melanoma is simply \( 1 - P(\text{Spitz}) \).

### Table 1

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Mean</th>
<th>Estimate of ( \alpha )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kanter-Lewensohn et al</td>
<td>46</td>
<td>4.00</td>
<td>0.250</td>
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<tr>
<td>Nagasaka et al</td>
<td>11</td>
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<td>39</td>
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<td>Li et al</td>
<td>8</td>
<td>6.90</td>
<td>0.145</td>
</tr>
<tr>
<td>Bergman et al</td>
<td>25</td>
<td>0.59</td>
<td>1.69</td>
</tr>
<tr>
<td>Chorny et al</td>
<td>12</td>
<td>2.80</td>
<td>0.357</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Mean</th>
<th>Estimate of ( \beta )</th>
</tr>
</thead>
<tbody>
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<td>Saenz-Santamaria et al</td>
<td>10</td>
<td>15.0</td>
<td>0.133</td>
</tr>
<tr>
<td>Fogt et al</td>
<td>8</td>
<td>20.1</td>
<td>0.0995</td>
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<tr>
<td>Boni et al</td>
<td>34</td>
<td>5.17</td>
<td>0.387</td>
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<tr>
<td>Kanter-Lewensohn et al</td>
<td>50</td>
<td>29.7</td>
<td>0.0673</td>
</tr>
<tr>
<td>Talve et al</td>
<td>35</td>
<td>14.4</td>
<td>0.139</td>
</tr>
<tr>
<td>Niezabitowski et al</td>
<td>26</td>
<td>21.2</td>
<td>0.0943</td>
</tr>
<tr>
<td>Nagasaka et al</td>
<td>93</td>
<td>11.4</td>
<td>0.175</td>
</tr>
<tr>
<td>Kaleem et al</td>
<td>32</td>
<td>16.1</td>
<td>0.124</td>
</tr>
<tr>
<td>Li et al</td>
<td>24</td>
<td>23.7</td>
<td>0.0842</td>
</tr>
<tr>
<td>Korabikowska et al</td>
<td>6</td>
<td>14.2</td>
<td>0.141</td>
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<tr>
<td>Miracco et al</td>
<td>20</td>
<td>40.2</td>
<td>0.0498</td>
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<tr>
<td>Bergman et al</td>
<td>26</td>
<td>27.7</td>
<td>0.0722</td>
</tr>
<tr>
<td>Chorny et al</td>
<td>12</td>
<td>25.3</td>
<td>0.0791</td>
</tr>
</tbody>
</table>

* N is the number of cases used for the reported mean, which is the mean of the proliferation index (percentage of tumor nuclei staining positive for MIB-1); \( \alpha \) is the parameter for the exponential probability density function and is estimated as 1/mean; \( \beta \) is the parameter for the \( \gamma \) probability density function and is estimated as 2/mean (see the “Materials and Methods” section).
Results

The results are given in Table 2, which provides calculated predictive probabilities using Equation 2 and unit intervals in restricted ranges of PI. The entries in Table 2 demonstrate that when the PI is less than 2%, Spitz nevus is more likely than melanoma. Also, when PI exceeds 6%, melanoma is more likely than Spitz nevus unless the estimate of P(\text{Spitz}) is high, and when PI exceeds 10%, Spitz nevus becomes unlikely regardless of P(\text{Spitz}). Between PI values of 2% and 6%, the probability that a lesion is a Spitz nevus depends significantly on the chosen value of P(\text{Spitz}). In this proliferation range and depending on P(\text{Spitz}), the tabulated predictive probabilities vary from as low as 0.11 up to 0.97, and the predicted probabilities would vary more had I used values for P(\text{Spitz}) of less than 0.25 or higher than 0.90.

Discussion

The article demonstrates that if one uses the MIB-1 PI to help discriminate between Spitz nevus and melanoma, the predictive probability that the lesion is a Spitz nevus depends on 4 variables: (1) the a priori probability that the lesion is a Spitz nevus (ie, P(\text{Spitz})); (2) the observed MIB-1 PI in the lesion; (3) the distribution function of PIs in Spitz nevi; and (4) the distribution function of PIs in malignant melanomas. Only when all 4 are known can one logically use the MIB-1 PI to estimate the predictive probability that the lesion is a Spitz nevus.

Of course the decision to use the MIB-1 PI in this differential diagnosis is a personal one for many pathologists and one that reflects the laboratory’s ability to execute an effective stain for MIB-1. In the absence of the MIB-1 PI, the final predictive probability that a lesion is a Spitz nevus is P(\text{Spitz}). In other words, the starting point for the algorithm using MIB-1 PI is the final point for reaching a diagnosis without using MIB-1. Thus, choosing an appropriate value for P(\text{Spitz}) should be straightforward. All one need do is use the routine clinical and histologic information about the lesion and routine guidelines to place the lesion into one of several categories such as the following: very likely to be a Spitz nevus (eg, P(\text{Spitz}) = 0.9); probably a Spitz nevus (eg, P(\text{Spitz}) = 0.75); unlikely to be a Spitz nevus (eg, P(\text{Spitz}) = 0.25); or unclear (ie, P(\text{Spitz}) = 0.5). Should this number of categories be too rigid, the methods and formulas in this article can be revised to include additional or other values of P(\text{Spitz}).

Although measuring the MIB-1 PI in a lesion might seem tedious, it is worth the effort and requires only a few minutes to execute. First, one must make sure that the stain has worked by using external and internal control cells. Internal control cells in the specimen that should be positive for MIB-1 include basal cells and lymphocytes. At the same time one must take care to exclude these cells from the count of tumor cells. One then should count several hundred tumor nuclei of cells in the dermis and in a systematic rather than a targeted manner, because this is how the data in Table 1 were obtained. Using a 65× objective and an eyepiece that is partitioned into quadrants can help divide this task into smaller parts, but neither is necessary. Finally, one expresses the result as the number of positive nuclei times 100 divided by the total number counted to yield a PI percentage.

Table 2

<table>
<thead>
<tr>
<th>PI Interval</th>
<th>P(\text{Spitz})</th>
<th>0.90</th>
<th>0.75</th>
<th>0.50</th>
<th>0.25</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-1</td>
<td>1.00</td>
<td>0.99</td>
<td>0.98</td>
<td>0.96</td>
<td></td>
</tr>
<tr>
<td>1-2</td>
<td>0.99</td>
<td>0.97</td>
<td>0.91</td>
<td>0.78</td>
<td></td>
</tr>
<tr>
<td>2-3</td>
<td>0.97</td>
<td>0.92</td>
<td>0.79</td>
<td>0.56</td>
<td></td>
</tr>
<tr>
<td>3-4</td>
<td>0.94</td>
<td>0.83</td>
<td>0.62</td>
<td>0.35</td>
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<tr>
<td>4-5</td>
<td>0.87</td>
<td>0.70</td>
<td>0.43</td>
<td>0.20</td>
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<tr>
<td>5-6</td>
<td>0.77</td>
<td>0.53</td>
<td>0.27</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>6-7</td>
<td>0.63</td>
<td>0.37</td>
<td>0.16</td>
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<tr>
<td>7-8</td>
<td>0.48</td>
<td>0.23</td>
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<td>0.03</td>
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<tr>
<td>8-9</td>
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<td>9-10</td>
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<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

PI, proliferation index.

* The MIB-1 PI is given in units of the percentage of cells staining; P(\text{Spitz}) is the a priori estimate for the probability that a lesion is a Spitz nevus and is based on histologic and clinical findings other than the proliferation rate. For example, values of P(\text{Spitz}) equal to 0.9 or 0.75 occur when the initial findings favor Spitz nevus, and a P(\text{Spitz}) value of 0.5 occurs when the diagnoses of Spitz nevus and melanoma seem equally likely. A P(\text{Spitz}) value of 0.25 occurs when the initial findings favor melanoma.
Although this study contributes nothing new regarding the estimate of \( P(\text{Spitz}) \) or the measurement of the MIB-1 PI, what it uniquely provides are its distribution functions for MIB-1 PIs in Spitz nevi and melanomas, its consolidation of the results of previous studies, and its algorithm for assembling the 4 key variables to estimate the predictive probability that a lesion is a Spitz nevus. Up to this point there has been no way to consolidate previously published results on PIs to yield guidelines for the impact of PIs in the differential diagnosis of Spitz nevi and melanomas. The derived exponential and \( \gamma \) distribution functions do this and rely on more than 140 cases of Spitz nevus and more than 400 cases of melanoma, so that they incorporate a magnitude of experience that none of us alone is likely to have. On the other hand, the entries for \( \alpha \) and \( \beta \) in Table 1 demonstrate considerable variation, which, in turn, implies uncertainty for the predictive probabilities in Table 2. For this reason, the entries for Table 2 should be considered guidelines that reflect average behavior rather than that of exceptional lesions. Furthermore, for every tabulated probability of Spitz nevus, there is a chance of melanoma. For every observed PI, there is a chance that the lesion might be a Spitz nevus or a melanoma. Such considerations simply underscore the probabilistic nature of the differential diagnosis between Spitz nevus and melanoma, as suggested by Piepkorn in 1995. Regardless of whether one uses MIB-1, the discrimination of Spitz nevus from melanoma is a probabilistic exercise, because for many spitzoid tumors the final diagnosis includes some uncertainty.

### Appendix 1

#### Maximum Likelihood Estimators for \( \alpha \) and \( \beta \)

For a series of measurements following an exponential probability density, the likelihood function, \( L \), is:

\[
L = \prod \alpha \times \exp(-\alpha \times x_i)
\]

where the product is taken over all values of \( x_i \) with \( i = 1, 2, \ldots, n \) and with \( n \) being the number of study cases. To find the maximum likelihood estimator for \( \alpha \), one first evaluates the natural logarithm of \( L \) (symbolized as \( \ln L \)), then differentiates \( \ln L \) with respect to \( \alpha \), sets this value equal to 0, and finally solves for \( \alpha \). Thus, \( \ln L \) is given as:

\[
\ln L = \sum \ln(\alpha) - \alpha \times \sum x_i
\]

Performing the differential and setting this to 0 yields:

\[
0 = n/\alpha - \sum x_i
\]

and solving for \( \alpha \) gives the estimate as \( n/\sum x_i \), which is 1/mean of \( x \).

For a series of measurements following a \( \gamma \) probability density, the likelihood function, \( L \), is:

\[
L = \prod \beta^2 \times x_i \times \exp(-\beta \times x_i)
\]

where the product is taken over all values of \( x_i \) with \( i = 1, 2, \ldots, n \) and with \( n \) being the number of study cases. As above, to find the maximum likelihood estimator for \( \beta \), one first evaluates the natural logarithm of \( L \) (symbolized as \( \ln L \)), then differentiates \( \ln L \) with respect to \( \beta \), sets this value equal to 0, and finally solves for \( \beta \). Thus, \( \ln L \) is given as:

\[
\ln L = \sum 2 \times \ln(\beta) + \Sigma x_i - \beta \times \sum x_i
\]

Performing the differential and setting this to 0 yields:

\[
0 = n \times \sum x_i - \sum(2/\beta \times x_i)
\]

and solving for \( \beta \) gives the estimate as \( n \times 2/\sum(2/\beta \times x_i) \), which is 2/mean of \( x \).

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**References**


**From Laboratory Medicine, Veterans Affairs Medical Center, and Department of Pathology, Duke University Medical Centers, Durham, NC.**

Address reprint requests to Dr Vollmer: Laboratory Medicine 113, VA Medical Center, 508 Fulton St, Durham, NC 27705.


