**β₂-Microglobulin**

A Reliable Parameter for Differentiating Between Graft Rejection and Severe Infection After Cardiac Transplantation

Harald Teufelsbauer,* Friedrich C. Prischl, MD,*
Michael Havel, MD, Christoph Holzinger, MD, Thomas Lion, MD,
Joseph D. Schwarzmeier, MD, and Axel Laczkovics, MD

We investigated the role of β₂-microglobulin as a noninvasive parameter to monitor acute rejection and severe infection in 45 consecutive heart transplant recipients. Endomyocardial biopsy revealed moderate (41 patients) or severe (three patients) rejection in 44 patients. Severe infections of bacterial septicemia (11 patients), bronchopneumonia (two patients), and viral infection (seven patients) were detected by a meticulous schedule of various clinical and laboratory tests. β₂-Microglobulin levels in serum, generally corrected for serum creatinine, were significantly elevated in patients with infections (median, 6.3 mg/l; range Q10–Q90, 3.47–10.27 mg/l) compared with levels in patients with rejection (p<0.0001) or in patients in obviously good condition (p<0.0001). At the onset of acute rejection, the median corrected β₂-microglobulin serum level was 1.56 mg/l (range Q10–Q90, −0.05–3.46 mg/l) and was significantly different from the control group (p<0.01). In addition, density function and empirical quantile analyses allowed us to define ranges of β₂-microglobulin levels that would differentiate between rejection (2.05–3.46 mg/l) and infection (>3.46 mg/l). With these values, sensitivity and specificity were 0.9 and 0.938 for detection of infection and 0.23 and 0.925 for detection of rejection, respectively. By means of β₂-microglobulin, two cases of infection were misinterpreted as rejection (10%), and four of 44 rejections were mistaken for infections (9%). We conclude that measurements of β₂-microglobulin may improve the management of heart transplant patients. (Circulation 1989;80:1681–1688)

Since the introduction of cyclosporine A as a potent immunosuppressive drug, cardiac transplantation has become part of the standard treatment in end-stage heart failure.1,2 The long-term outcome, however, depends on the effective management of acute allogeneic rejections and severe infections that have remained the main complications. Therefore, numerous studies deal with the early and reliable diagnosis of these complications. For detection of rejection, hemodynamic,3 electrophysiologic,4 and various immunologic parameters5,6 are under investigation; nevertheless, endomyocardial biopsy still is the reference standard for definite diagnosis.7

The monitoring of infection requires the accurate and meticulous recording of various clinical and laboratory parameters. Despite these efforts, some life-threatening infections, for example, those induced by cytomegalovirus, give rise to conditions that are confused with periods of acute rejection.

Recently, several investigators have shown that serum levels of β₂-microglobulin were significantly increased before clinically detectable rejection in renal transplant patients.8,9 Similar findings were described in cardiac transplantation.10 Furthermore, various immunologic disorders including infections have been reported to be accompanied by abnormal levels of β₂-microglobulin in serum.11–14 This 11,600-Da protein is present on the surface of all cells expressing major histocompatibility complex class I antigens and is structurally related to the light chain of this antigen class.15,16 Because stimulation of the immune system also leads to enhanced major histocompatibility complex I expression, β₂-microglobulin shedding might be increased too and measurable changes of β₂-microglobulin in both rejection and infection might be expected.15,17

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*The first two authors have made equal contributions to this manuscript.
From the 2nd Department of Surgery (H.T., M.H., C.H., A.L.) and the 1st Department of Medicine (F.C.P., T.L., J.D.S.), University of Vienna, Austria.
Address for correspondence: Dr. F.C. Prischl, 3rd Department of Medicine, Krankenhaus Wels, Grieskircherstrasse 42, A-4600 Wels, Austria.
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Thus, we investigated the usefulness of serum β₂-
microglobulin measurements to diagnose and differ-
entiate these two states in 45 patients who had undergone cardiac transplantation.

Methods

Patients

From March 1984 to September 1987, 45 consec-
tutive patients (43 males, two females) underwent
heart transplant because of end-stage heart failure
and were enrolled in this study. The mean age was
41.1 years (range, 6–56 years). Cardiac transplan-
tation was performed by the classic orthotopic
 technique. The postoperative immunosuppressive
regimen consisted of cyclosporine A, azathioprine,
and methylprednisone as described previously. In
addition, antithymocyte globulin was given during
the first 10 days.

After transplantation, the patients were examined
daily for 1 month. Then, for 2 months, routine
check-ups were performed every 2 weeks or whenever
subjective complaints or suspicious clinical
findings required further control. Afterward, the
intervals between controls ranged from each week
to every 3 months depending on the individual
patient’s condition. Routine check-ups included clin-
ical status, electrocardiography, chest radiograph,
cholecardiography, and laboratory tests (liver- and
kidney-function parameters, leukocyte count, eryth-
rocyte and platelet counts, cytoimmunologic moni-
toring, neopterin, β₂-interferon, and others).

As of September 1987, 19 of the 45 patients died.
Death was caused by infection or rejection in six
patients each. The others died from acute myocard-
ial infarction (three patients), right heart failure
(one), multiorgan failure (one), or cyclosporine A-
duced heart failure (two, Reference 19). Six deaths
occurred within the first month after transplantation
because of rejection (two patients), infection (two),
right heart failure (one), and multiorgan failure
(one). The other 13 patients died after a median
time of 4 months because of rejection (four patients,
53±11 days), infection (four, 135±75 days),
cyclosporine-induced heart failure (two, 141±99
days), and myocardial infarction (three, 420±45
days; median±median absolute deviation).

Monitoring of Acute Rejection

Endomyocardial biopsy was performed once a
week during the first postoperative month and every
2 weeks during the following 2 months. Later,
biopsy was performed in variable intervals as
described above but immediately whenever rejec-
tion was suspected.

Overall, 366 biopsies were performed and eval-
uated histologically according to Billingham’s stag-
ing, that is, grade 0: no evidence of rejection;
grade 0–1, 1: mild; grade 1–2, 2: moderate; and
grade 2–3, 3: severe pathologic changes.21 Acute
rejection was defined as changes of grade 1–2 or
more in the biopsy sample. According to this
definition, 44 rejection episodes (grade 1–2, 2: 41
episodes; grade 2–3, 3: three episodes) were diag-
nosed and treated with 1,000 mg methylpred-
nisone i.v. daily for 3 days.

Monitoring of Infection

A rigorous schedule of tests was applied to detect
infections. In addition to daily clinical and labora-
tory examinations described above, once weekly
blood and urine samples were cultured during the
first month. Every 3 days, sputum samples were
collected, and a smear of the sample was analyzed.
Weekly, complement binding reactions and immu-
nofluorescence techniques specific for herpes sim-
plex and cytomegalovirus (CMV) were performed on
blood, urine, and sputum samples. After the first
month, these tests were performed at every routine
check-up.

Definition of severe infection was occurrence of
clinical symptoms of systemic infection and posi-
tive results of blood cultures (Streptococcus epider-
midis excluded), positive herpes- or CMV-antigen,
or pneumonia (confirmed by radiography) with pos-
tive sputum culture. Bacterial septicemia was found
in 11 patients and bronchopneumonia in two (Table
3). In three patients, obvious clinical symptoms of
infection were seen and CMV antigen and antibod-
ies were detected in blood, sputum, or cerebrospi-
nal fluid. Four patients had herpes simplex infec-
tions, one of whom (patient 10, Table 3) had fever
for 1 day only and developed herpes labialis with-
out further signs of systemic manifestation. Asympt-
omatic bacteriuria or asymptomatic but positive
results of sputum cultures were excluded from
evaluation. Patients were treated with different anti-
biotic regimens according to the respective antibio-
grams. Patients with CMV infections received CMV
hyperimmunglobulin (Cytotect) and gancyclovir
intravenously, and patients with herpes received
acyclovir intravenously until clinical reconstitution.

β₂-Microglobulin

β₂-Microglobulin levels were measured in serum
once before heart transplant and daily during the first
postoperative month. After this period, measure-
ments were obtained at any routine check-up. Over-
all, 1,736 β₂-microglobulin determinations were
performed using a commercially available radio-
immunoassay (betamicro RIA, Pharmacia, Upps-
sala, Sweden). Because β₂-microglobulin levels in
serum may be altered by impaired renal function,23
nominal serum levels of β₂-microglobulin for given
serum creatinine levels were estimated with the fol-
lowing equation:

\[
\ln \text{serum } \beta_2 \text{-microglobulin (mg/l)} = 3.834 - 5.96 \times y + 2.94 \times y^2 - 0.476 \times y^3 + 0.0252 \times y^4
\]

where \( y = \ln \text{serum creatinine (mg/l)} \) and with
\( r = 0.9849 \).
The difference between these estimated and the actually measured 1g2-microglobulin levels was calculated and termed “corrected 1g2-microglobulin.” This was used for further statistical evaluation. As a condition sine qua non, 1g2-microglobulin values were selected for analysis only if reference values of 1g2-microglobulin sampled immediately before the rejection or infection events were within the normal range. In fact, this was true for all events in this study.

**Statistical Analysis**

Three subgroups, that is, patients with acute rejection, with severe infections, and with no evidence of both these complications were statistically analyzed. The latter (standard) group was established by a random selection of 20 1g2-microglobulin determinations using normally distributed random numbers (Table 2). The median and median absolute deviation were calculated, and box plots were created to show approximate differences between the three groups. Furthermore, Q10 and Q90 quantiles and empirical density plots (see Reference 25 and Appendix) were computed and drawn for more precise illustration. After these calculations, threshold levels, that is, the Q90 quantiles, were obtained for clinical testing.

To quantify the significance of differences between medians the Mann-Whitney rank sum test was used to obtain p values. The significance level was p < 0.01. The null hypothesis was equality of the medians of the compared groups; the alternate hypothesis was inequality. The results were controlled by the median rank test. Statistics were computed by SAS and locally available PLI programs with an IBM 3700.

To evaluate the clinical relevance of threshold levels obtained by the methods mentioned above, sensitivity, specificity, and predictive values were calculated (see Appendix). Sensitivity-specificity plots were drawn to confirm the suggested threshold as the best compromise between these two parameters.

As shown in Tables 1, 2, and 3, several patients were included more than once in the same group or in different groups. Thus, the results could be seriously biased by a patient who responded peculiarly to multiple rejections or infections. Therefore, multiple analyses were performed as follows. One infection-associated sample was randomly selected for 10 of the 17 patients with infection (Table 3). For another 10 patients, who were not in the first group but who had a rejection episode, one rejection associated sample was randomly selected (Table 1). Finally, for 10 more patients not in either of these groups, one normal sample was randomly selected (Table 2). Then, the Q10 and Q90 values along with the associated sensitivity and specificity were computed. The entire process was repeated 10 times, and average Q10, Q90, and sensitivity and specificity values were obtained. Finally, these median values were used to classify all 20 cases of infection and 44 cases of rejection.

**Results**

**Rejection**

The peak level of 1g2-microglobulin observed within a maximum of 7 days before biopsy was defined as

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**Table 1.** β2-Microglobulin Values in Serum of 22 Cardiac Transplant Patients With Graft Rejection (n=44)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum β2-microglobulin (mg/l)</th>
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<td>8</td>
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<td>0.95</td>
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<td>9</td>
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</tr>
<tr>
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<td>30</td>
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</tr>
<tr>
<td>31</td>
<td>1.97</td>
<td>5.77</td>
</tr>
</tbody>
</table>

Median±MAD: 1.09±0.24 3.67±1.24 2.02±0.57 1.56±0.84

MAD, median absolute deviation.
the equivalent of rejection (Table 1). This peak level preceded diagnosis of rejection by biopsy at an average of 3 days. After correction for serum creatinine, the median of peak levels was 1.56 mg/l (range Q10–Q90, −0.05–3.46 mg/l) and differed significantly (p<0.01) from the standard group in which the median was 0.46 mg/l (range Q10–Q90, −0.33–2.045 mg/l). Box plots are shown in Figure 1. The β₂-microglobulin levels in rejection did not correlate with pathologic grading.

Figure 2 illustrates the empirical densities of the corrected β₂-microglobulin levels. The curve characteristics of the standard group with its median near zero show the validity of the correction of β₂-microglobulin for serum creatinine. Despite the statistically significant difference between the rejection and standard group (p<0.01), the overlap in density function curves seemed to be too large to achieve adequate clinical differentiation between the two groups. However, by computing Q90 of both collectives, a numerical range of 2.046–3.46 mg/l could be established as indicative of rejection. With these values, sensitivity and specificity for detection of rejection were 0.227 and 0.925, respectively. The positive predictive value was 0.769, and the negative predictive value was 0.521.

**Infection**

In 17 patients, 20 cases of infection were diagnosed (Table 3). All patients had fever, and the

### Table 2. β₂-Microglobulin Values in Serum of Cardiac Transplant Patients Without Evidence of Rejection or Infection (Standard)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum β₂-microglobulin (mg/l)</th>
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</tr>
<tr>
<td>45</td>
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</tr>
</tbody>
</table>

Median±MAD 1.14±0.32 3.09±1.15 2.15±0.73 0.46±0.36

**MAD**, median absolute deviation.

### Table 3. β₂-Microglobulin and Creatinine in Serum of Cardiac Transplant Patients With Severe Infections (n=20)

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Infectious agent</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum β₂-microglobulin (mg/l)</th>
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<td></td>
<td></td>
<td>Measured</td>
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<td>12.80</td>
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<tr>
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<td>Cytomegalovirus</td>
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<td>17.00</td>
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<tr>
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<td>1.90</td>
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<td>7.30</td>
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<tr>
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<td>Pseudomonas aeruginosa</td>
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<td>11.80</td>
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</tr>
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<td>19.40</td>
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<tr>
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<tr>
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</tr>
<tr>
<td></td>
<td>Pseudomonas aeruginosa</td>
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<td>12.13</td>
</tr>
</tbody>
</table>

Median±MAD 1.2±0.34 8.3±3.34 2.3±0.89 6.3±1.29

**MAD**, median absolute deviation.
mean white blood cell count was 7.73 G/l (range, 2.6–15.9 G/l). In contrast to patients with rejection, the highest β₂-microglobulin values were found in patients with infection during the ongoing infectious process. For evaluation, the value of the day when infection became clinically obvious was used, and the median value was 6.3 mg/l (range Q10–Q90, 3.47–10.27 mg/l; Figure 1). On computation of p values, a remarkable difference was found between the infection group and the standard (p<0.00001) and the rejection group (p<0.00001).

Figure 2 shows the minor overlap between β₂-microglobulin in rejection and infection. Within our patients, only two cases of infection with false-negative results were seen (10%). In both cases, herpes simplex was found. In one patient with systemic cutaneous herpes and fever, the β₂-Microglobulin level of 3.35 mg/l (patient 11, Table 3) was borderline to the defined threshold. The second patient (patient 10, Table 3) had moderate fever for 1 day but developed herpes labialis only without any signs of systemic infection. β₂-Microglobulin remained normal. In rejection, four of 44 (9%) episodes were accompanied by corrected β₂-microglobulin values in the “infection range” (>3.46 mg/l). Defining β₂-microglobulin values above 3.46 mg/l (Q10 of the infection group) as the equivalent of infection sensitivity and specificity for detection of infection were 0.9 and 0.938, respectively. The validity of this threshold level between rejection and infection was also confirmed by multiple sensitivity and specificity calculations at different β₂-microglobulin values (Figure 3). The positive and negative predictive values were 0.81 and 0.97, respectively, the latter indicating the high clinical relevance to exclude severe infections.

Of note, infections caused by CMV were accompanied by remarkably increased β₂-microglobulin levels, that is, 12.37, 7.22, and 6.48 mg/l. The highest level was found in a patient with manifest CMV encephalitis. In the two other patients (CMV pneumonitis, CMV septicemia), the corrected β₂-microglobulin levels even further increased with disease progression (8.07 and 8.3 mg/l). In each case, the increase in corrected β₂-microglobulin levels could be observed even before diagnosis was definitely established (median, 2 days). Similar findings have been described by others.¹³

**Statistical Cross-Validation of Results**

The multiple analyses of randomly selected, independent samples of the infection, rejection, and standard group (see “Methods”) confirmed the results as shown above. The median Q10 of the

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**Figure 1.** Box plots of β₂-microglobulin values (mg/l), corrected for serum creatinine, in cardiac transplant recipients. The boxes represent 50% of the actual β₂-microglobulin values of each group. The horizontal line in the box indicates the median. The vertical line above and below the box comprises the range between Q10 and Q90. The rings represent values outside these quantiles.

**Figure 2.** Plot of empirical density (boxcar functions) of β₂-microglobulin levels (mg/l). After correction for serum creatinine, the β₂-microglobulin levels (mg/l). After correction for serum creatinine, the β₂-microglobulin values of the cardiac transplant patients without complications (standard, 20 patients), with rejection (44 patients), or with severe infection (20 patients) were used to compute empirical density (see Appendix) of the three groups.
infection associated samples was 3.485 mg/l of corrected β2-microglobulin. The median Q90 of the standard samples was 2.051 mg/l. Thus, the respective ranges were ≤2.051 for the standard group, 2.052–3.484 for the rejection group, and ≥3.485 mg/l for the infection group. Average sensitivity and specificity for infection were 0.95 and 0.95, respectively. In rejection, the average sensitivity and specificity were 0.24 and 0.935, respectively. The classification of all 20 episodes of infection and 44 cases of rejection according to subranges derived from multiple computations did not differ from the overall analysis described above. Therefore, sensitivity and specificity values remained unchanged.

**Discussion**

The long-term outcome after heart transplant is no more a matter of operation techniques but rather depends on effective postoperative care with sensitive and reliable diagnosis of impending graft rejection and severe infection. Many noninvasive tests have been proposed to detect acute rejection, but endomyocardial biopsy is still the only method that combines high sensitivity with acceptable specificity. To some extent, the measurement of β2-microglobulin has a similar disadvantage to other immunologic tests because it reflects an unspecific activation of the immune system. However, as we have shown, precise subranges of this parameter specific for infection or rejection could be defined. After correction for possibly diminished renal function, β2-microglobulin levels ranging from 2.045 to 3.46 mg/l were highly suggestive of an ongoing rejection. They may force one to immediately perform endomyocardial biopsy. β2-Microglobulin values above 3.46 mg/l clearly pointed to severe infection.

Though overall sensitivity for detection of rejection was low, only four episodes of rejection showed β2-microglobulin levels above the defined range and could have been mistaken for infection. Excluding the patient with herpes labialis (patient 10, Table 3), only one case of infection was misclassified as rejection. The ability to differentiate between the two states in such a precise manner (Figure 2) was due to the high specificity of the two corresponding β2-microglobulin ranges. In the four episodes of rejection classified erroneously as infection, rejection was accompanied by clinical signs of severe and prolonged hemodynamic alterations leading to edema and congestive hepatomegaly. The latter may cause diffuse alterations of liver cells and thus may contribute to the extraordinary increase in serum β2-microglobulin. Whether or not minor hemodynamic changes caused by rejection influence β2-microglobulin levels has yet to be elucidated.

In infection, our results on corrected β2-microglobulin values revealed not only high specificity but also high sensitivity for the detection of life-threatening infections. The 20 episodes of severe infections were accompanied by highly elevated β2-microglobulin levels with only two false-negative results. In both cases, the causative pathogen was herpes simplex virus. In one patient after 1 day of fever, a local herpes labialis developed only. In the other patient who had prolonged fever and systemic cutaneous manifestations, the corrected β2-microglobulin level was borderline below the defined threshold (patient 11, Table 3). Similar observations are described by Edwards et al. The conclusion may be that only severe systemic infections are a potent stimulus for the immune system and thus may generate a remarkable increase of β2-microglobulin levels. The course of β2-microglobulin values during CMV infections supports this view. All three patients with severe CMV infections had markedly increased β2-microglobulin values before diagnosis by conventional tests. Our observation may gain importance in patients with atypical pneumonia and (false?) negative results of CMV antigen tests. Fine needle puncture might possibly be avoided in such cases.

The pathophysiologic basis for the increase in β2-microglobulin levels at the beginning of rejections and during infections is still not clarified, but two mechanisms may be assumed. In the first mechanism, the production of β2-microglobulin may...
be enhanced in parallel with an increased major histocompatibility complex class I antigen expression caused by T-interferon. This may represent a crucial event in the rejection process and is also supported by the fact that histologic changes and clinical diagnosis of rejection are remarkably preceded by the increase in serum $\beta_2$-microglobulin.

In the second mechanism, elevated $\beta_2$-microglobulin values may be due to increased cell damage with subsequent liberation of membrane-associated $\beta_2$-microglobulin. Interestingly, different courses of $\beta_2$-microglobulin levels were observed in patients with infection or rejection. In rejection, an early peak in $\beta_2$-microglobulin with following tendency to decrease was seen, whereas in infection, the $\beta_2$-microglobulin levels increased in parallel to the progressive infectious process. A possible explanation is that rejections may be induced by a temporary imbalance of activating and suppressive factors within the immune system, whereas infections may represent a more continuous process with a steady state-like continuity of activating factors.

In conclusion, the measurement of $\beta_2$-microglobulin in serum (under consideration of the corresponding serum creatinine) seems to be a helpful tool for the fast diagnosis of severe infection. Furthermore, $\beta_2$-microglobulin levels offer clinically useful information for the indication to perform endomyocardial biopsy and also to reduce the frequency of biopsy. This may be helpful especially in severely ill patients. Further prospective studies will have to prove our encouraging results.

**Appendix**

Calculation of clinically predictive values:

- Sensitivity = TP/(TP + FN)
- Specificity = TN/(TN + FP)
- Positive predictive value = TP/(TP + FP)
- Negative predictive value = TN/(TN + FN)

where TP is true positive: positive result in a patient with disease, TN is true negative: negative result in a patient without disease, FP is false-positive: positive result in a patient without disease, and FN is false-negative: negative result in a patient with disease.

Calculation of empirical density function:

$$f(x) = \frac{1}{b \cdot n} \cdot \sum w(\frac{x - x_i}{b})$$

where boxcar function $w(u)$ is 1 if $|u|$ is less than or equal to 0.5 and is 0 if $|u|$ is greater than 0.5, $n$ is number of observations, $b$ is length of the interval $(x-0.5b, x+0.5b)$, $x_i$ is observations in the actual interval, and $x$ is equidistant locations (freely eligible—the $x$ range must include the whole $x_i$ range).

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**References**


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