Patterns of urinary β_2-microglobulin excretion by patients treated with aminoglycosides

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Patterns of urinary β_2-microglobulin excretion in patients treated with aminoglycosides. Aminoglycoside antibiotics are relatively mild nephrotoxins, but their action is site-specific to the proximal tubule. Therefore, use of these drugs presents a unique opportunity to study the temporal relation between the damage to the cells lining the renal proximal tubule and the subsequent rise in the serum creatinine concentration. Our study of 52 aminoglycoside-treated patients included measurements of daily serum creatinine, daily 24-hour urinary β_2-microglobulin (β_2M) excretion, and determination of aminoglycoside tissue accumulation. An elevation in β_2M excretion above the baseline value occurred in 37 of 52 (71%), whereas the serum creatinine concentration rose in only 17 of 52 (33%) of patients. Even fewer patients (10 of 52) demonstrated all three criteria for aminoglycoside nephrotoxicity. These 10 patients had elevated tissue accumulation, evidence of renal tubular damage, and a rise in serum creatinine concentration. The increased β_2M excretion greater than 50 mg/day preceded the serum creatinine rise by 2 to 7 days. An abnormal baseline β_2M was not a risk factor for a subsequent rise in creatinine concentration or vice versa. Although each test is primarily site specific, widespread and severe renal proximal tubular damage, regardless of cause, will eventually lead to an elevation of serum creatinine. Thus, serial monitoring of proximal tubular function with urinary β_2M excretion has potential value in the assessment of insults to this site, but cannot be expected to explain all changes in serum creatinine.

Modalités de l'excrétion urinaire de β_2-microglobulines chez les malades traités par les aminoglycosides. Les aminoglycosides ont une action néphrotoxique de sévérité moyenne spécifiquement localisée au tube proximal. L'emploi de ces drogues offre l'opportunité d'étudier la relation dans le temps entre la lésion des cellules tubulaires proximales et l'élévation consécutive de la créatininémie. L'étude de 52 malades traités par les aminoglycosides a comporté la détermination quotidienne de la créatininémie et de l'excrétion urinaire de β_2-microglobuline (β_2M) ainsi que la détermination de l'accumulation tissulaire d'aminoglycoside. Une élévation de l'excrétion de β_2M au dessus de la valeur basale a été observée chez 37 malades (71%) alors que l'élévation de la créatinine n'est survenue que chez 17 malades (33%). Dix malades seulement ont eu les trois critères de néphrotoxicité par l'aminoglycoside. Ces 10 malades avaient une accumulation tissulaire, des signes de lésions tubulaires et une élévation de la créatinine. Une augmentation de l'excrétion de β_2M, au dessus de 50 mg/24 hr a précédé de 2 à 7 jours l'élévation de la créatininémie. Une excrétion basale anormale de β_2M n'est pas un facteur de risque d'élévation ultérieure de la créatininémie, ou réciproquement. Bien que chacun de ces tests soit spécifique d'un site, des lésions tubulaires proximales sévères et étendues doivent déterminer une élévation de la créatinine, quelle que soit leur cause. La surveillance itérative de la fonction tubulaire proximale par la mesure de l'excrétion urinaire de β_2M a une valeur potentielle dans l'évaluation des lésions mais ne peut pas expliquer la totalité des modifications de la créatininémie.

Aminoglycoside antibiotics cause dose-related damage to the cells lining the renal proximal tubule [1, 2]. Their nephrotoxicity is best understood in relation to their pharmacokinetic disposition, as all aminoglycosides accumulate to high concentrations in the renal cortex [3–5] and persist there for weeks to months following cessation of treatment [6–9]. Patients treated with these agents are monitored by measuring either their serum antibiotic or their serum creatinine concentrations, but rising or excessive serum creatinine levels are late markers of aminoglycoside nephrotoxicity in both man [10, 11] and animals [1, 2, 12].

It has been proposed that following an insult to the renal tubules, the glomerular filtration decreases in response to a failure of renal proximal tubular reabsorptive capability [13–17]. Aminoglycoside nephrotoxicity is specific to the renal proximal tubule, and although the mechanism is unknown, patients with aminoglycoside-induced nephrotoxicity demonstrate a 3- to 7-day lag time between early damage and subsequent rise in serum creatinine concentration [10, 18–20]. Because this lag time allows observation of several stages of damage, aminoglycoside antibiotics, as relatively mild renal proximal tubular toxins, offer a unique opportunity

to study the sequence of events between renal tubular damage and the decline in glomerular filtration.

Aminoglycoside-associated renal tubular damage occurs even in patients with normal baseline renal function [10, 21, 22]. Of greater concern, the damage can occur also in patients whose dosages are adjusted to maintain "safe" peak and trough drug concentrations [10, 11, 22]. Because close monitoring of blood aminoglycoside and serum creatinine concentrations may not always prevent renal damage, an urgent clinical need exists for a more sensitive test that reflects the proximal tubular site of damage. Damage to renal tubules during therapy can be monitored by urinary enzyme excretion [18, 23, 24], urinary cast excretion [19, 25], or by the urinary loss of small molecular weight proteins, such as β_2-microglobulin (β_2M) [26, 27]. All of these indices are more specific and earlier indicators of renal tubular damage than is the serum creatinine concentration [10, 27]. None of these renal tubular indices, however, can distinguish aminoglycoside effects from other clinical insults [10, 19, 24, 25, 27].

In the absence of a specific test for aminoglycoside nephrotoxicity, a choice among the available methods for monitoring damage to the renal proximal tubule might be made on relative sensitivity. Before this decision can be made, however, correlation must be established between the urinary excretion of each of the tubular indices and the subsequent rise in serum creatinine concentration. To this end, we evaluated 52 seriously ill patients by daily 24-hour urine collections for β_2M excretion. Our goals were to assess the value of β_2M in the detection of the early stages of renal tubular damage that lead to the elevation of serum creatinine concentration, and to establish the level of urinary β_2M excretion that would serve as a critical value in the prediction of a subsequent creatinine rise.

Methods

Fifty-two patients (29 males, 23 females) aged 65 ± 14 years, were treated 9.5 ± 5.5 days with aminoglycoside antibiotics for serious gram-negative infections. Of the 52 patients, 18 (35%) were given gentamicin, 27 (52%) were given tobramycin, and 7 (13%) were given amikacin. Dosages of these agents were adjusted to yield therapeutic peak and trough serum concentrations by means of nomograms and serum assays. All patients were treated in intensive care units for pneumonia, septicemia, or abdominal infections complicating major surgery, or for infections complicating exacerbation of major medical disorders, including congestive heart failure, diabe-

tes, liver disease, gastrointestinal bleeding, and chronic lung disease. In addition to aminoglycoside therapy, β-lactam antibiotics or clindamycin were also given, concurrently, to most patients with infections. Of the 52 patients, 21 were given cephalosporins, and 36 (69%) were given furosemide.
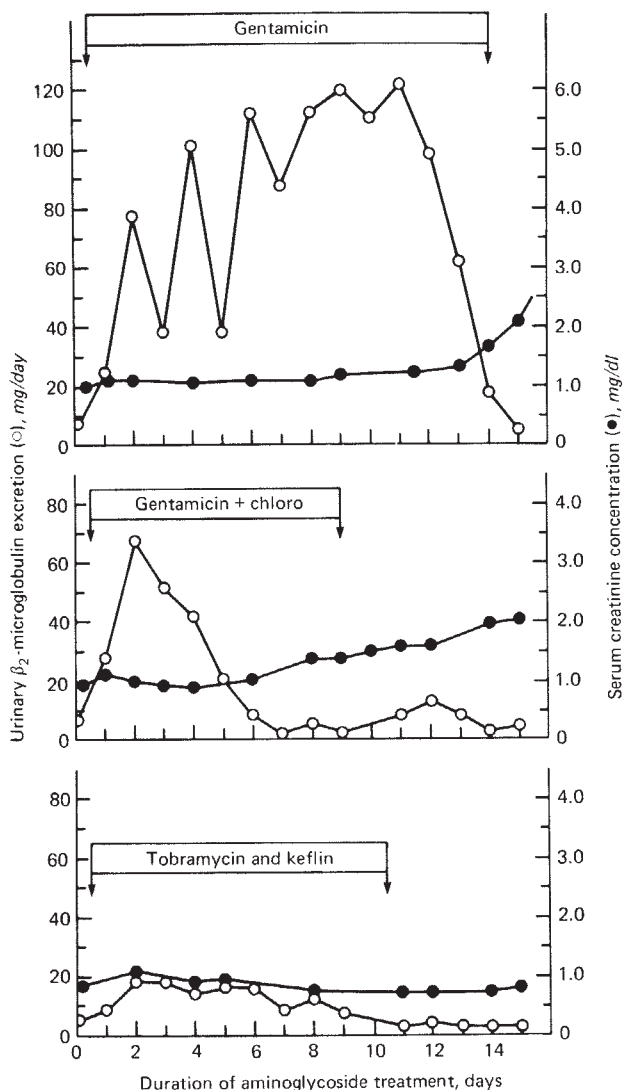
The serum creatinine concentration was measured daily by standard autoanalyzer methods, and the upper limit of normal was 1.5 mg/dl for this method. The pretreatment serum creatinine concentration averaged 1.4 ± 0.9 in the 52 patients, and the 24-hour creatinine clearance values averaged 61 ± 20 ml/min. These values indicate mild renal disease in most patients and likely reflect the advanced age of our 52 patients. A further serum creatinine concentration increase of 0.5 mg/dl was defined as a significant change in glomerular filtration.

Urinary β_2M excretion was measured before treatment and daily from 24-hour urine collections, and was assayed with a commercially available radioimmunoassay (Pharmacia, Piscataway, New Jersey). All urine samples were alkalinized with 1.0 N sodium hydroxide to prevent β_2M degradation prior to assay [28]. Values were plotted versus time to evaluate patterns of excretion and changes in excretion rate. The baseline period was the 1- to 2-day period before treatment was started. Urinary β_2M excretion was considered normal if it was below 1.0 mg/day [26, 27]. Elevations in β_2M were defined as increases of 1.0 mg/day or greater above baseline.

Aminoglycoside serum concentrations were measured by radioimmunoassay (Monitor Science, Newport Beach, California). Therapeutic peak and trough serum concentrations were considered to be 10.0 and 2.0 μg/ml for gentamicin and tobramycin, and 25.0 and 6.0 μg/ml for amikacin. Aminoglycoside tissue accumulation was calculated from pharmacokinetic analysis with a two-compartment model, as previously described [29]. High values for aminoglycoside tissue accumulation were those amounts above 200 mg for gentamicin, above 175 mg for tobramycin, and above 600 mg for amikacin [10, 30]. Tissue accumulation was confirmed by analysis of aminoglycoside urinary recovery after the final dose, or by postmortem tissue analysis [5, 6, 10, 29, 30]. The majority of the calculated tissue accumulation is found in the kidney, and the proximal tubular concentrations are probably reflective of this calculated value [5, 29].

Results

Patterns of excretion and order of magnitude. Inspection of the daily β_2M values in each of the 52

Fig. 1. *Three typical patterns of β₂M excretion noted in this study. Open circles* represent the *β₂M* excretion rate, and *closed circles* represent the serum creatinine concentration. The aminoglycoside and concurrent antibiotics given are indicated on the graph. All values are plotted versus time in days.

patients revealed several distinct temporal patterns of β_2M excretion. The three most frequently encountered patterns are illustrated in Fig. 1.

A patient with a marked β_2M response to aminoglycoside exposure is shown in the top frame. The β_2M excretion rapidly increased to above 50 mg/day, continued to rise over the entire course of gentamicin treatment, and declined only as serum creatinine concentration began to rise rapidly. Of the 52 study patients, 8 demonstrated a similar β_2M pattern. Of the 8, 7 were elderly obese women, 5 of the 8 had diabetes, all received furosemide, and 6 were given, concurrently, cephalosporins. None of

the patients had positive blood cultures, severe dehydration, or shock.

A significant rise in serum creatinine concentration occurred in only 3 of these 8 patients, in spite of the multiple renal insults and the markedly elevated β_2M excretion. In these 3 patients, the β_2M excretion remained elevated until the serum creatinine concentration rose; then it decreased. In the other 5, β_2M fell to normal within 10 days following the last aminoglycoside dose.

Of the 52 patients, 21 had β_2M excretion patterns similar to the middle frame. Of these 21, 8 received cephalosporins, 14 received furosemide, 8 had diabetes, 8 had shock, and 8 had positive blood cultures. There were 11 males and 10 females; all were older patients with severe infections. Of these 21 patients, 10 had an increase in serum creatinine concentration. Of these 10, 7 had β_2M excretions above 50 mg/day. Of the remaining 11 patients without creatinine rise, 6 also had β_2M excretions above 50 mg/day.

The pattern of β_2M excretion for the remaining 23 patients is shown at the bottom of Fig. 3. This pattern is qualitatively similar to that noted in the top frame, but the excreted amount of β_2M is far lower. These 23 patients were apparently more tolerant of aminoglycoside treatment, but did not differ from the other two groups in clinical presentation. Of these 23, 7 received cephalosporins, 14 received furosemide, 9 had diabetes, 6 had shock, and 5 had positive blood cultures. There were 15 males and 8 females; all were elderly patients with complicated infections. Only 4 of these 23 patients had a significant rise in serum creatinine concentration, and of these 4, only 1 had a creatinine rise that was preceded by elevated β_2M excretion above 50 mg/day. In the group of 23 patients at the bottom of Fig. 3, only 2 had β_2M elevations above 50 mg/day. One patient experienced a β_2M elevation without a serum creatinine rise, and the other had an increase in serum creatinine preceded by a β_2M rise above 50 mg/day.

Most of the 52 patients reached this peak β_2M excretion in the first 4 days of aminoglycoside treatment, as shown at the top of Fig. 2, whereas a small number of patients required as many as 8 to 11 days. The majority of patients also had peak excretion values below 25 mg/day, with a few individuals excreting up to 225 mg/day, as shown in the center frame of Fig. 2. Finally, β_2M values in most patients returned to baseline values within 1 to 3 days after the last dose of aminoglycoside.

Changes in serum creatinine. All 52 patients were initially evaluated in terms of their baseline serum
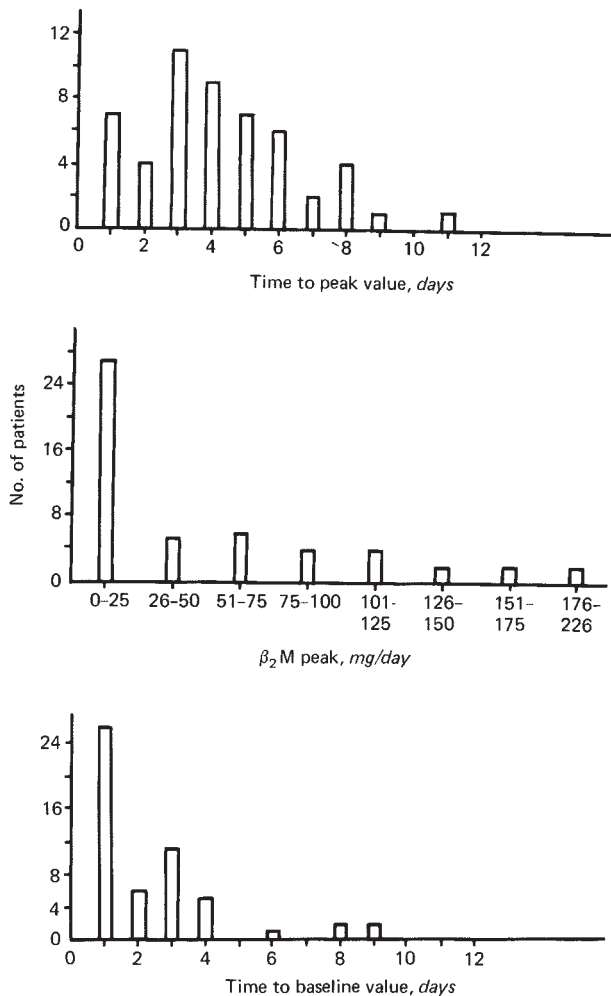
Fig. 2. *Classification of the 52 study patients under time to peak excretion* (top), *magnitude of peak excretion* (center), *and time to return to baseline values after cessation of treatment* (bottom).

creatinine concentrations. As shown in Fig. 3, of the 52 patients, 13 (25%) had baseline serum creatinine concentrations above the laboratory range for normal. During aminoglycoside treatment, 6 patients with elevated baseline creatinine concentrations had further creatinine increases, compared to 11 patients with normal baseline values. The differences are not significant ($\chi^2 = 1.42$, P = NS).

During treatment, elevations of serum creatinine concentrations were noted in fewer study patients than were elevations of β_2M. Of the 52 patients, 17 (33%) had an elevation in creatinine concentration, but 37 (71%) had an increase in β_2M excretion above the normal range. Rises in β_2M always occurred prior to the serum creatinine rise, but a β_2M rise was not always followed by an elevation in the serum creatinine during aminoglycoside treatment (Fig. 3).

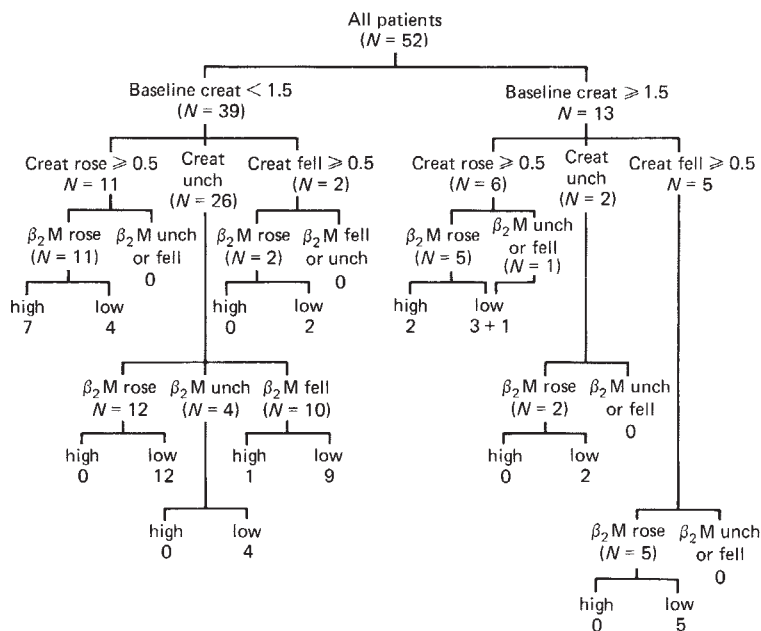*Prediction of serum creatinine rise from β_2M rise.* The baseline β_2M excretion and subsequent changes in the 52 patients are illustrated in Fig. 4. Thirty-one patients (60%) had baseline β_2M above, and 21 patients had baseline values below 1.0 mg/day. The baseline elevations of most patients were mild, as only 13 of the 31 patients with abnormal β_2M baseline values had β_2M values above 10.0 mg/day. During aminoglycoside therapy, 37 patients had a β_2M increase over the baseline values, and patients with β_2M rises were equally divided between baseline groups, as shown in Fig. 4. Because these differences were not significant ($\chi^2 = 0.43$, P = NS), no greater incidence of β_2M rise occurred in patients with abnormal baseline β_2M values. Only 5 of the 52 patients had a normal baseline β_2M concentration that remained unchanged, which demonstrates the almost universal renal tubular effects of disease or treatment in these seriously ill patients.

Figure 4 also shows the serum creatinine response of the study patients during aminoglycoside therapy. Seventeen study patients (31%) had a serum creatinine rise during aminoglycoside treatment, but these 17 patients showed no differences between β_2M baseline groups in their subsequent incidence of creatinine rise ($\chi^2 = 1.65$, P = NS). As such, an elevated baseline β_2M cannot be considered a risk factor for a further creatinine rise in patients given aminoglycosides.

In contrast to the patients with a β_2M rise from baseline, only 1 of the 15 patients with falling or unchanged β_2M excretion had a creatinine elevation. This was true whether or not the β_2M concentration was abnormal at the baseline measurement. Therefore, a low stable β_2M excretion, or a decreasing β_2M excretion rate is unlikely to herald a later rise in serum creatinine concentration in aminoglycoside treated patients (Fig. 4).

In summary, most patients with a β_2M concentration rise did not have a subsequent rise in their serum creatinine concentration. Furthermore, an abnormal β_2M baseline in aminoglycoside-treated patients was not more predictive of a subsequent creatinine rsie than was a normal β_2M baseline value.

Relations between β_2M and aminoglycoside tissue accumulation. The fourth division of both Figs. 3 and 4 shows data on accumulation of aminoglycoside in tissue. In Fig. 3, a high tissue accumulation was found in 2 patients with abnormal baseline serum creatinine concentrations and in 8 patients with normal baseline serum creatinine concentrations. In 9 of these 10 patients, this ele-

All patients
(N = 52)

Baseline creat < 1.5
(N = 39)

Baseline creat ≥ 1.5
N = 13

Creat rose ≥ 0.5   Creat   Creat fell ≥ 0.5   Creat rose ≥ 0.5   Creat   Creat fell ≥ 0.5
N = 11   unch   (N = 2)   (N = 6)   unch   N = 5
                        (N = 26)   (N = 2)

β_2M rose β_2M unch β_2M rose β_2M fell β_2M rose β_2M unch
(N = 11)   or fell   (N = 2)   or unch   (N = 5)   or fell
                  0   0   (N = 1)

high   low   high   low   high   low
7   4   0   2   2   3 + 1

β_2M rose β_2M unch β_2M fell β_2M rose β_2M unch
N = 12   (N = 4)   (N = 10)   (N = 2)   or fell
                                                                              0

high   low   high   low   high   low
0   12   1   9   0   2

high   low β_2M rose β_2M unch
0   4   (N = 5)   or fell
                                                                                       0

                                                                     high   low
                                                                     0   5

Fig. 3. *Grouping of the 52 study patients based on baseline serum creatinine values.* The *first division* classifies the baseline serum creatinine concentration, the *second* documents whether the serum creatinine concentration rose, fell, or was unchanged (unch) during aminoglycoside treatment. The *third* division gives β_2M changes preceding the change in serum creatinine, and specifically refers to changes in β_2M from baseline through the aminoglycoside treatment period. The *fourth* division (unlabeled) gives aminoglycoside tissue accumulation during treatment. High values were above 200 mg for gentamicin, above 175 mg for tobramycin, and above 600 mg for amikacin.

vated tissue accumulation was observed in association with a rise in both urinary β_2M excretion and in serum creatinine. These 9 patients fit our criteria for aminoglycoside nephrotoxicity [10, 30]. The tenth patient had a creatinine rise of 0.4 mg/dl, and is also likely to have had aminoglycoside nephrotoxicity.

Tissue accumulation in association with a baseline β_2M excretion is illustrated in Fig. 4. Of the 31 patients with abnormal baseline β_2M, 8 demonstrated high tissue accumulation, whereas high tissue accumulation was noted in 2 of the 21 patients with normal baseline β_2M excretion. Because this trend is not statistically significant ($\chi^2 = 2.14$, $P =$ NS), baseline tubular dysfunction does not predispose patients to abnormally high tissue accumulation, at least in situations where aminoglycoside blood levels are maintained within the recommended range.

Comparing the tests for serum creatinine, urinary β_2M, and tissue accumulation, our data indicate the greatest sensitivity to be for β_2M, as 71% of these aminoglycoside-treated patients has a β_2M rise, compared with 33% having a creatinine rise, and only 10% having an elevated tissue accumulation. Because only half of the patients with both renal tubular damage and elevated serum creatinine concentrations had elevated tissue accumulation, we
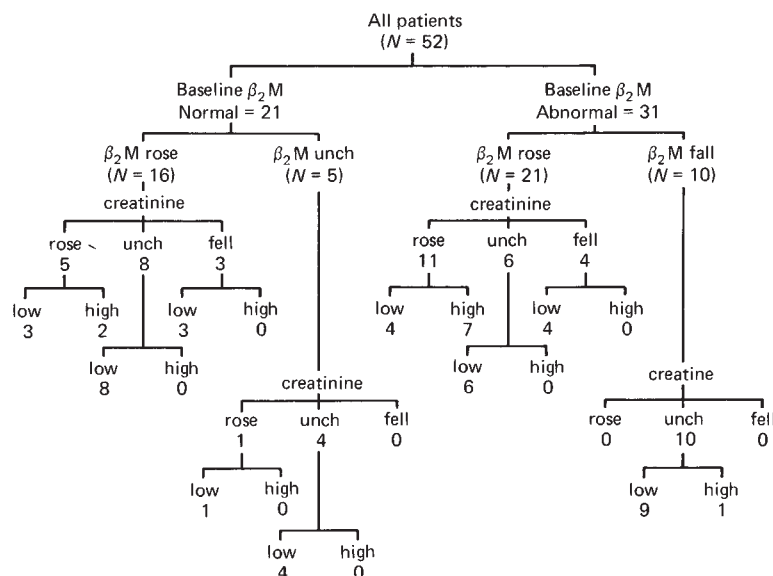
postulated that only half of the treated patients with an elevation in serum creatinine had aminoglycoside-related renal damage. In the remainder, we ascribed the renal damage primarily to the clinical condition, or possibly as having been aggravated by the aminoglycoside treatment.

Thus, in the assessment of aminoglycoside nephrotoxicity, β_2M is a very sensitive but not specific test. Elevated tissue accumulation is most specific, yet difficult to assess routinely. The measurement of serum creatinine is insensitive and nonspecific, but universally available.

Discussion

The reabsorptive function of the healthy renal proximal tubule includes the active uptake and catabolism of small-molecular-weight proteins such as β_2-microglobulin (β_2M) [26, 28, 31, 32]. Reabsorption of filtered β_2M is so efficient that normal urinary losses are below 1.0 mg/day [27, 28].

After filtration by the glomerulus, β_2M is bound to the brush border membrane of the proximal tubule, and high concentrations of basic amino acids such as lysine will act as a competitive inhibitor [33]. Competitive binding at the membrane level has been demonstrated between aminoglycosides and lysine, but only at lysine levels sufficient to saturate

All patients
(N = 52)

Baseline β_2M
Normal = 21

Baseline β_2M
Abnormal = 31

β_2M rose
(N = 16)
creatinine

β_2M unch
(N = 5)

β_2M rose
(N = 21)
creatinine

β_2M fall
(N = 10)

rose 5　unch 8　fell 3

rose 11　unch 6　fell 4

low 3　high 2

low 3　high 0

low 4　high 7

low 4　high 0

low 8　high 0

creatinine

rose 1　unch 4　fell 0

low 6　high 0

creatine

rose 0　unch 10　fell 0

low 1　high 0

low 9　high 1

low 4　high 0

Fig. 4. *Grouping of the 52 study patients based on their baseline β_2M values.* The *first division* classifies patients based on normal or abnormal baseline β_2M. An abnormal baseline β_2M was above 1.0 mg/day. The *second* division gives the course of β_2M excretion during the aminoglycoside treatment period. Values either rose, fell, or remained unchanged. The *third* division gives the resulting course of serum creatinine during the aminoglycoside treatment. Values either rose ≥ 0.5 mg/dl, fell ≥ 0.5 mg/dl, or remained stable (fluctuations between ± 0.5 mg/dl). The *fourth* division (unlabeled) gives the aminoglycoside tissue accumulation in each of the patients. High values were above 200 mg for gentamicin, above 175 mg for tobramycin, and above 600 mg for amikacin.

the lysine reabsorptive site [34]. Competitive inhibition could occur between β_2M and aminoglycosides as well, because we have noted that β_2M excretion rate fluctuates in the interval between aminoglycoside doses (unpublished observations). Because of these fluctuations, the β_2M excretion rate must be quantitated in terms of the amount per day to interpret values. Competition for reabsorptive sites alone, however, is an unlikely explanation for the increased β_2M loss in aminoglycoside-treated patients, because even though the daily filtered load of β_2M is similar to the filtered amount of aminoglycoside, far more β_2M molecules are reabsorbed.

Because structural damage to the proximal tubule is minimal after 1 to 3 days of treatment [2, 20, 35], failure of β_2M reabsorption could also be considered an early defect in tubular function, which would lead to structural damage as the drug exposure continued. If urinary β_2M were a marker of reabsorptive function, any insult that impairs the ability of the proximal tubule to reabsorb small-molecular-weight proteins might also result in an elevated β_2M excretion rate [36-38]. It may require a more severe renal tubular insult to elevate the concentration of creatinine, because 71% of aminoglycoside-treated patients had some elevation of β_2M, but only 33% had a rise in serum creatinine.

The patient population chosen for our study undoubtedly includes a higher percentage with renal tubular damage than would be found in a randomly selected group of hospitalized patients. β_2M rose in almost all aminoglycoside-treated patients. These β_2M increases might be common, because the drugs aggravate the renal tubular functions of many older critically ill patients. Alternatively, the increases may reflect the sensitivity of this test to detect trivial impairment in proximal tubular functions.

Our study suggests that a β_2M excretion of 50 mg/day is appropriate to define renal tubular damage in most situations. No chosen β_2M value, however, can be expected to predict all serum creatinine elevations, because the serum creatinine concentration might also rise when the GFR changes without prior renal tubular damage.

β_2M is readily measured by means of a sensitive and commercially available radioimmunoassay. Its extreme sensitivity to defects in proximal tubular function allows quantitation of subtle changes induced by drugs and environmental toxins [36-38]. Although β_2M is not a specific test for aminoglycoside nephrotoxicity, this same disadvantage is also shared by renal enzymes, cast excretion, and all other methods devised to monitor drug effects on the renal tubule. Serum creatinine, other tests of glomerular filtration, and aminoglycoside trough levels are even less specific for tubular damage than is β_2M, and are also less sensitive. These markers of glomerular filtration merely confirm, ret-

rospectively, that tubular nephrotoxicity has occurred.

Our serial use of β_2M has revealed its exceptional sensitivity, and also has provided insight into the renal tubular insults that occur in acutely ill patients given aminoglycosides. The use of this test reveals subtle changes in tubular function of most aminoglycoside-treated patients, and its potential value in the study of proximal tubular dysfunction remains an important area for further exploration.

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