Urinary Excretion of β2-Microglobulin and IgG Predict Prognosis in Idiopathic Membranous Nephropathy: A Validation Study

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An accurate prediction of the prognosis of patients with idiopathic membranous nephropathy (iMN) should allow restriction of immunosuppressive treatment to patients who are at highest risk for ESRD. On the basis of retrospective studies, it has previously been suggested that the urinary excretions of β2-microglobulin (Uβ2m) and IgG (UIgG) are useful predictors of renal insufficiency in patients with iMN. The threshold values of 0.5 μg/min (Uβ2m) and 250 mg/24 h (UIgG) have been validated in a new and larger patient cohort. From 1995 onward, 57 patients with iMN (38 men, 19 women; age 48 ± 16 yr), a nephrotic syndrome, and a serum creatinine level ≤1.5 mg/dl were studied prospectively. At baseline, a standardized measurement was carried out to determine renal function and protein excretion. The end point renal death was defined as a serum creatinine exceeding 1.5 mg/dl or a rise of serum creatinine of >50%. Mean (±SD) follow-up was 53 ± 23 mo. Thus far, 25 (44%) of the patients have reached the end point renal death. Multivariate analysis confirmed Uβ2m as the strongest independent predictor for the development of renal insufficiency. Sensitivity and specificity were 88 and 91%, respectively, for Uβ2m, and both were 88% for UIgG. When the excretions of both proteins were combined, specificity improved to 97%. It is concluded that the present data validate the accuracy of Uβ2m and of UIgG in predicting renal outcome in patients with iMN. These markers can be used to guide decisions on the start of immunosuppressive treatment.

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Idiopathic membranous nephropathy (iMN) is one of the most frequent causes of the nephrotic syndrome in adults (1). If left untreated, up to 40% of patients will progress to ESRD (2–4). The efficacy of immunosuppressive therapy has been demonstrated in a randomized, controlled trial (4). Although this study provided arguments to treat all patients with iMN and a nephrotic syndrome, most authors advocate restricting immunosuppressive treatment to patients who are at highest risk for developing ESRD (5,6). It is well established that deterioration of renal function is a powerful predictor of ESRD (7,8). Therefore, a trial of immunosuppressive therapy is warranted in patients with iMN and established renal insufficiency. However, it is evident that immunosuppressive treatment started at a relatively late time point may be less effective in attaining normal renal function (9). Moreover, we and others have noted that the use of immunosuppressive agents in patients with renal insufficiency was associated with more frequent and more severe side effects than in patients who are treated in an earlier phase of their disease (10–12). Therefore, it would be ideal if treatment could be optimized by identifying high-risk patients at an earlier time point. In patients with iMN, various risk factors for the development of renal failure have been identified (13). However, the sensitivity and the specificity of most of these factors (*e.g.*, age, gender, glomerular injury, tubular interstitial fibrosis) are too low to justify their use to guide decisions on the start of immunosuppressive therapy. Thus far, the level and the duration of proteinuria are the best predictive factor in a model introduced by the Toronto Glomerulonephritic Registry (14). This model requires a minimal observation period of 6 to 18 mo.

On the basis of data derived from small patient cohorts, we demonstrated previously that the urinary excretion of β2-microglobulin (Uβ2m) and IgG (UIgG), assessed in a single urine sample, independently predicted the development of renal insufficiency in patients with iMN (15,16). Our data suggested high sensitivities and specificities, which ranged from 80 to 90%. We now have validated these results in a prospectively studied, new and larger patient cohort.

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Materials and Methods

In our center, patients with proteinuria are evaluated using a standard protocol. In all of these patients, standardized urine and blood measurements are carried out as described below. For the validation study, we prospectively studied patients with biopsy-proven iMN, evaluated from 1995 onward. In the analysis, we included only patients with a baseline serum creatinine ≤1.5 mg/dl and proteinuria ≥2.7 g/g creatinine and/or serum albumin ≤3.0 g/dl. We excluded patients who had been treated with immunosuppressive drugs other than oral prednisone. Patients were also excluded when the interval between renal biopsy and the baseline measurement exceeded 3 yr.

Standardized Measurement of Urinary Proteins

Patients come to the ward after an overnight fast. Patients are instructed to take 4000 mg of sodium bicarbonate on the evening before to ensure that urinary pH exceeds 6.0, which is mandatory for the measurement of Uβ2m. On the morning of the measurement, patients are not allowed to take diuretics. Upon arrival, 375 to 500 ml of tap water is given to enforce diuresis. The patients remain supine during 2 h except for voiding. BP measurements are done using an automatic device, and 10 consecutive readings are registered with an interval of 5 min (DINAMAP; Criticon, Tampa FL). Timed urine samples are collected, and in the middle of the collection period, a blood sample is drawn. In addition, two 24-h urine samples are collected for assessment of daily excretion of total protein and creatinine.

Laboratory Measurements

In the blood samples, we assessed the following parameters: Creatinine, cholesterol, β2m, albumin, IgG, and transferrin. In the timed urine samples, we measured creatinine, β2m, α1-microglobulin, albumin, IgG, and transferrin. The concentrations of serum creatinine, serum cholesterol, urinary total protein, and urinary creatinine were measured with standard automated techniques. The concentrations of albumin, transferrin, α1-microglobulin, and IgG in serum and urine were measured by immunonephelometry on a BNII nephelometer (Behring, Marburg, Germany) using antibodies whose specificity was checked by Ouchterlony double immunodiffusion and immunoelectrophoresis (Dako, Glostrup, Denmark). Urinary and serum β2m were measured by ELISA as described before (17).

Calculations

Endogenous creatinine clearance (ECC) was calculated according to the formula Ucr × V/Pcr, where Ucr is the concentration of creatinine in the urine, V is the urine flow, and Pcr is the plasma concentration of creatinine, and was corrected for body surface area. Because 24-h urine samples were not collected regularly during follow-up, we estimated creatinine clearances using the Cockcroft and Gault formula. In addition, we calculated GFR for patients who reached the end point renal death by applying the recently developed Modification of Diet in Renal Disease (MDRD) formula using serum creatinine, age, gender, race, serum albumin, and serum urea (18). The mean arterial pressure was the average of the last six of 10 registered measurements.

The amounts of β2m, α1-microglobulin, IgG, transferrin, and albumin in the timed urine samples are expressed as excretion per unit time (minute or 24 h). Protein selectivity index was calculated as the clearance of IgG divided by the clearance of transferrin. The total protein excretion in the 24-h urine samples was expressed as g/g creatinine to correct for sampling errors.

Statistical Analyses

For the validation study, we calculated renal survival using Kaplan-Meier statistics. Renal death was defined as an increase of serum creatinine >50% or an increase of serum creatinine >1.5 mg/dl. Survival was calculated using the date of the baseline study at $t = 0$. We compared renal survival using log-rank test for patients with low and high Uβ2m and UIgG. We used the threshold values established in our previous studies (15,16). The threshold level for β2m excretion was 0.5 μg/min and for IgG was 250 mg/24 h. Using these threshold levels, we calculated sensitivity, specificity, true positive predictive value, and true negative predictive value.

Because the use of a fixed serum creatinine value as end point, irrespective of the baseline value, might have introduced a bias (a subtle increase in serum creatinine could have been defined as failure), we performed a subanalysis in a group of patients with a baseline serum creatinine <1.2 mg/dl.

Using the data of the present patient cohort, we also studied the effect of other parameters in predicting renal outcome. Univariate analysis and multivariate analysis using the Cox proportional hazard model with a forward stepwise procedure was performed to identify independent predictive parameters. Receiver operating characteristics (ROC) curves were made to determine the area under the curve (AUC) and to calculate the sensitivity and the specificity using the most discriminative thresholds. The following parameters were plotted into ROC curves: β2m excretion, IgG excretion, α1-microglobulin excretion, transferrin excretion, albumin excretion, selectivity index, ECC, serum creatinine, serum albumin, and total proteinuria per 24 h. The parameters with the highest AUC were selected and used as covariates in the Cox regression analysis. All values are given as means (±SD) or medians (range) when appropriate. All statistics were performed using SPSS software, version 11.0 (Chicago, IL). $P < 0.05$ was considered significant.

Results

From 1995 to 2002, we studied 57 patients who had iMN and fulfilled the inclusion criteria. In 90% of the patients, the baseline measurement was performed within 1 yr after renal biopsy. Baseline characteristics are given in Table 1. Two patients had been treated with prednisone. Patients have been followed for 53 ± 23 mo. Thus far, 25 (44%) patients have reached the predefined end point of renal death. The reason for renal death was a serum creatinine >1.5 mg/dl in 21 patients and a rise of >50% of serum creatinine in 4 patients. Overall renal survival was 81% at 6 mo, 68% at 1 yr, and 54% at 3 yr. Thus, in most patients, progressive disease was apparent within 3 yr after the baseline study. In this new patient cohort, the use of the previously established threshold values of Uβ2m and UIgG excretion allowed an accurate prediction of renal outcome. Renal survival curves are depicted in Figures 1 and 2. Our calculations confirmed the high sensitivity and specificity (Table 2). We evaluated the possible bias of using the fixed serum creatinine value of 1.5 mg/dl as end point. To this end, we assessed the extent of the deterioration of renal function. In the 25 patients who reached the predefined end point of renal death, serum creatinine had increased by an average of 46% from 1.15 ± 0.2 to 1.65 ± 0.24 mg/dl. Calculated creatinine clearance (Cockcroft and Gault formula) was 76 ± 22 ml/min per 1.73 m^2 at baseline and 52 ± 13 ml/min per 1.73m^2 at the end point. The absolute decrease of creatinine clearance averaged 45 ml/min per 1.73 m^2/yr. For comparison, in the nonfailure group, the average change of calculated creatinine clearance was 1.7 ml/min per 1.73 m^2/yr. When we estimate GFR using the recently

Table 1. Baseline characteristics of patients with iMN ($n = 57$)[a]

Gender (M/F)	38/19
Age (yr)	48 ± 16
MAP (mmHg)	98 ± 16
ECC-24 h (ml/min per 1.73 m^2)	88 ± 26
Serum creatinine (mg/dl)	1.00 ± 0.23
Serum β2m (mg/L)	2.8 ± 1.1
Serum albumin (g/dl)	2.4 ± 0.5
Cholesterol (mg/dl)	329 ± 76
Interval Bx measurement (mo)	2 (0–33)
Follow-up[b] (mo)	53 ± 23
Timed urine sample:	
albumin excretion (mg/min)	3.8 (0.3–16)
IgG excretion (mg/24 h)	197 (18–3597)
β2m excretion (μg/min)[c]	0.38 (0.05–68.4)
α1m excretion (μg/min)	29 (4–418)
transferrin excretion (μg/min)	283 (17–1455)
selectivity index	0.18 (0.06–0.39)
proteinuria (g/g creatinine)	5.8 (1.7–13.3)

[a]Data are means ± SD or medians (range). IMN, idiopathic membranous nephropathy; MAP, mean arterial pressure; ECC 24 h, creatinine clearance calculated from 24 h urine; β2m, β2-microglobulin; Bx, renal biopsy.
[b]From baseline measurement until end of follow-up.
[c]In case of β2m excretion: $n = 56$; in one patient, β2m was not measurable because pH urine was too low (<6.0).

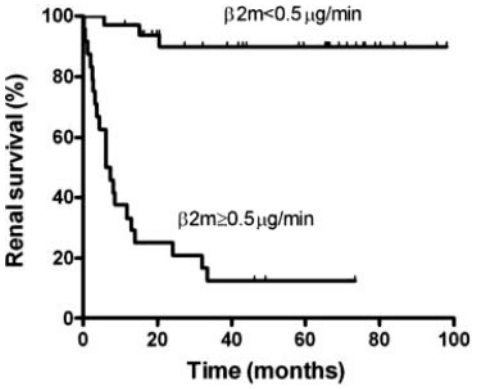

Figure 1. Renal survival in patients with idiopathic membranous nephropathy (iMN) with urinary β2-microglobulin excretion (Uβ2m) <0.5 μg/min and ≥0.5 μg/min. Renal death was defined as an increase of serum creatinine to values >1.5 mg/dl or an increase of serum creatinine >50%.

developed MDRD formula, the severity of renal dysfunction is even more manifest: The MDRD GFR at the predefined end point (and thus at the start of immunosuppressive therapy) was 37 ± 9 ml/min per 1.73 m^2. Of note, because we did not calibrate serum creatinine values against the standard of the MDRD reference laboratory, our calculated MDRD GFR may underestimate true GFR by 5 ml/min per 1.73 m^2.

The difference in course of renal function between patients with high and low Uβ2m can be appreciated by comparing the

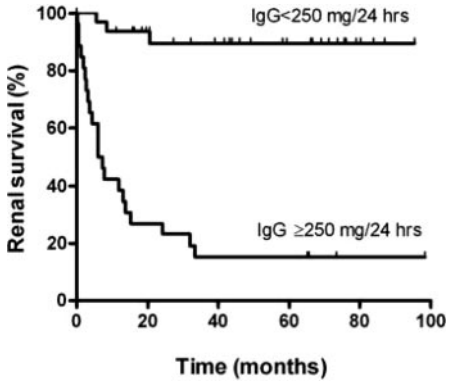

Figure 2. Renal survival in patients with iMN and an IgG excretion <250 mg/24 h *versus* patients with an IgG excretion ≥250 mg/24 h.

slopes of 1/serum creatinine: In patients with low Uβ2m, the slope was −0.012 dl/mg per yr (interquartile range, −0.04 to 0.014); in patients with high Uβ2m, the slope was −0.42 dl/mg per yr (interquartile range, −0.91 to −0.16; $P < 0.01$).

A subgroup analysis limited to 44 patients with an initial serum creatinine <1.2 mg/dl resulted in similar conclusions: Renal survival was 93% at 6 mo, 79% at 1 yr, and 67% at 3 yr. In this subgroup, 14 (32%) patients reached the end point of renal death; at baseline, their serum creatinine was 1.00 ± 0.14 mg/dl and increased by 63% to 1.64 ± 0.31 mg/dl before start of immunosuppressive therapy. In this subgroup analysis, both Uβ2m and UIgG predicted prognosis. Renal survival was 33% at 1 yr in patients with high Uβ2m and 97% in patients with low Uβ2m. Calculated sensitivity and specificity were 79 and 97% for the Uβ2m and 79 and 90% for the IgG excretion. The specificity improved to 100% when the β2m and IgG excretion were combined.

We also explored our data using all available parameters. In the initial multivariate analysis, α1-microglobulin was not included in view of the very high correlation between Uβ2m and urinary α1-microglobulin. In univariate analysis, the following parameters were significantly related to renal outcome: Serum creatinine ($P < 0.001$), serum albumin ($P < 0.001$), ECC ($P < 0.01$), proteinuria ($P < 0.001$), selectivity index ($P < 0.001$), and urinary excretion of albumin, β2m, α1-microglobulin, transferrin, and IgG (all $P < 0.001$). Multivariate analysis revealed that Uβ2m was the strongest independent predictive factor (relative risk, 1.030; 95% confidence interval, 1.017 to 1.043; $P < 0.001$), indicating that the risk for renal insufficiency increased by 3.0% for every 0.1 μg/min increase of Uβ2m. After Uβ2m, serum albumin was identified as the second independent predictive factor (relative risk, 0.786; 95% confidence interval, 0.691 to 0.894; $P < 0.01$).

We calculated sensitivity and specificity for the various parameters (Table 2). When combining parameters, specificity can be somewhat increased (Table 2). ROC curves, as depicted in Figure 3, confirmed the best performance of Uβ2m, as reflected by the AUC.

We specifically evaluated urinary α1-microglobulin excretion in comparison with β2m excretion. There was a high correlation

Table 2. Sensitivity, specificity, PPV, and NPV of the most discriminative threshold levels of urinary proteins and creatinine clearance in the prediction of renal failure[a]

Parameter	AUC	Threshold	Sensitivity	Specificity	PPV	NPV
Uβ2m	0.947	0.5 μg/min	88%	91%	88%	91%
UIgG	0.876	250 mg/24 h	88%	88%	85%	90%
Uα1m	0.956	40 μg/min	84%	94%	91%	88%
Uexc albumin	0.896	2.8 mg/min	92%	69%	70%	92%
Uexc transferrin	0.906	350 μg/min	80%	84%	80%	84%
Proteinuria	0.898	8 g/24 h	88%	72%	71%	89%
SI	0.687	0.16	76%	50%	54%	73%
ECC 24 h	0.741	80 ml/min per 1.73 m^2	64%	81%	73%	74%
Serum creatinine	0.833	1 mg/dl	76%	81%	76%	81%
Serum albumin	0.913	2.2 g/dl	80%	97%	95%	86%
Combinations						
high Uβ2m + high UIgG		0.5 μg/min + 250 mg/24 h	83%	97%	95%	89%
high Uβ2m + low serum albumin		0.5 μg/min + 2.2 g/dl	75%	100%	100%	84%
high Uα1m + high UIgG		40 μg/min + 250 mg/24 h	76%	94%	91%	83%
high Uα1m + low serum albumin		40 μg/min + 2.2 g/dl	72%	100%	100%	82%

[a]Uexc, urinary excretion; α1m, α1-microglobulin; SI, selectivity index; PPV, positive predictive value; NPV, negative predictive value.

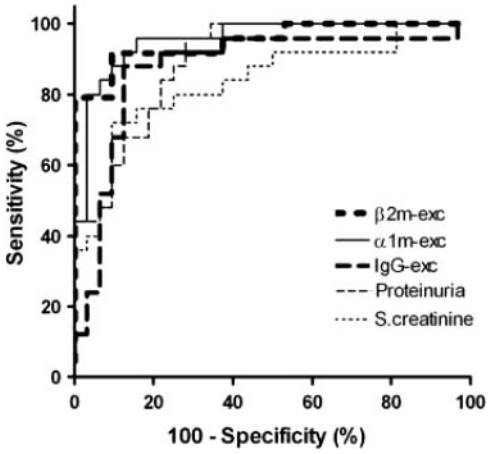

Figure 3. Comparative efficacy of serum creatinine and the urinary excretion of several proteins for predicting renal death in patients with iMN ($n = 57$). Receiver operating characteristics curves of β2m excretion (β2m-exc; area under the curve [AUC], 0.947), α1-microglobulin excretion (α1m-exc; AUC, 0.956), IgG excretion (IgG-exc; AUC, 0.876), proteinuria per day (AUC, 0.898), and serum creatinine concentration (s.creatinine; AUC, 0.833).

between these parameters ($r = 0.80$, $P < 0.001$). In fact, it is evident from Table 2 and Figure 3 that urinary α1-microglobulin excretion and Uβ2m give comparable results.

Discussion

We have validated the performance of Uβ2m and UIgG as predictors for renal insufficiency in patients with iMN. To this end, we tested the threshold values developed in our previous studies in a new, prospectively studied patient cohort. Our data clearly demonstrate that Uβ2m and UIgG predict with high accuracy renal outcome in patients with iMN. In fact, the calculated sensitivities and specificities are nearly identical to the values obtained in our previous studies (13). Thus, our data indicate that the model parameters are robust.

Our study may be criticized because we used a fixed value of serum creatinine of 1.5 mg/dl as end point for defining renal death. However, it is evident from calculated creatinine clearance and MDRD GFR that renal function was severely disturbed at the end point. The slope of 1/serum creatinine proved that there was a clear loss of renal function. Adopting a doubling of serum creatinine or 50% decrease of GFR as end point would have resulted in even longer withholding of immunosuppressive treatment.

We used a restrictive treatment policy in our patients, initiating immunosuppressive treatment as renal failure was evident. On the basis of the results of the randomized study conducted by Ponticelli *et al.* (4), one might ask whether delay of treatment is justified especially in patients with a nephrotic syndrome. Our treatment policy was based on our preliminary findings that immunosuppressive treatment with cyclophosphamide is effective in patients with established renal failure. We recently extended these observations and also demonstrated that a restrictive treatment policy results in excellent patient and renal survival rates (9,12).

In our previous study, we noted that the UIgG was the only variable that was independently associated with renal function deterioration. This superiority of UIgG over Uβ2m was explained by one patient in whom results of UIgG and Uβ2m did not concur. In this patient, who developed renal insufficiency, UIgG exceeded the threshold value of 250 mg/d, whereas Uβ2m was below the threshold (16). In our present, larger study cohort, Uβ2m was the most significant independent predictive factor. It has been well established that Uβ2m reflects

the severity of tubulointerstitial injury (19,20). Thus, our findings are in good agreement with studies that have unequivocally shown that in patients with glomerular diseases, renal outcome is more related to the presence and the extent of tubulointerstitial injury than to glomerular pathology. In general, there was a good agreement between Uβ2m and UIgG. When both parameters were combined, specificity even increased to a value of 97%.

How can we explain that UIgG and Uβ2m accurately predict renal failure? We propose that UIgG reflects the severity of glomerular damage, whereas Uβ2m is a marker of tubulointerstitial injury. It has been suggested that IgG or other high molecular weight proteins cause tubular cell activation or injury that results in tubulointerstitial inflammation, the final step toward renal insufficiency.

Thus far, only one model for the identification of patients who have iMN and are at risk for the development of chronic renal failure has been validated. The model was developed with data derived from the Toronto Glomerulonephritis Registry. In the first study, the duration and the level of proteinuria proved to be fairly accurate predictive factors. The best performance was found using a level of proteinuria >8 g/d for >6 mo. Calculated sensitivity was 66%, and specificity was 88% (13,14). In the validation study, roughly similar figures were reported with a sensitivity of 58% and a specificity of 93% (21). In addition, the Toronto group extended the model by calculating a risk score on the basis of the data of a selected 6-mo interval with the worst sustained proteinuria. In this model are included the minimum amount of proteinuria in that 6-mo interval, the initial creatinine clearance, and the slope of the creatinine clearance during the 6-mo period. The risk score model was validated in three different populations and proved quite good with sensitivities varying from 60 to 89%, specificities from 86 to 92%, and an overall accuracy of 79 to 87% (21). Obviously, this model has a very good performance. However, there are several disadvantages, particularly the need to have an observational period that exceeds a period of 6 mo and the necessity of multiple, accurate 24-h urine collections. Our model is based on the collection of a single timed urine sample collected in the morning period.

Furthermore, it is unproved whether the Toronto model can be applied to patients with newly diagnosed iMN. The model has been validated and applied to a group of patients with well-defined follow-up. This suggests that a long observation period was used to define the 6-mo period with the worst sustained proteinuria. In more than one quarter of the patients, the 6-mo period started >12 mo after renal biopsy. Therefore, the model may not be applicable to patients with a follow-up after biopsy of <12 to 18 mo.

In the present study, we specifically analyzed the value of urinary α1-microglobulin, a low molecular weight protein like β2m. In routine clinical practice, measurement of urinary α1-microglobulin is easier in view of its relative stability at pH <6.0. We observed a very high correlation between Uβ2m and urinary α1-microglobulin. Sensitivities and specificities were also comparable, although, admittedly, the threshold values used for α1-microglobulin should be validated in a second population. Our data confirm and strengthen the conclusion of Bazzi *et al.* (22). In a small cohort of 19 untreated patients with iMN, a nephrotic syndrome, and normal renal function, these authors found that urinary α1-microglobulin predicted the development of chronic renal failure with a sensitivity and a specificity of 100%. We applied their threshold value of 33.5 mg/g creatinine to our study cohort of 57 patients and calculated a sensitivity of 88% and a specificity of 78%. Bazzi *et al.* also reported the predictive value of UIgG. Using a threshold value of 110 mg/g creatinine, sensitivity was 100% and specificity was 69%. Applying this threshold value to our study cohort, we calculated a sensitivity of 92% and a specificity of 63%. We used a higher cutoff value (250 mg/d, approximately 180 mg/g creatinine), thereby increasing specificity. We believe that a high specificity should be pursued to be able to avoid unnecessary immunosuppressive therapy in patients with iMN.

The data of our secondary analysis suggest that serum albumin may have added value as a prognostic marker. Admittedly, this needs confirmation in another patient cohort. Can we avoid unnecessary immunosuppressive treatment by using Uβ2m and UIgG as prognostic markers? From our data, it can be calculated that when used in the present population with a failure rate of 44% (which is in close agreement with literature data), our established threshold values would have resulted in the unnecessary treatment of one patient (1.8% overall, 4.8% of all treated patients), whereas 31 patients rightly would not have received treatment.

Conclusion

We have validated the performance of Uβ2m and UIgG as prognostic markers in patients with iMN. Urinary α1-microglobulin can replace Uβ2m. Use of these markers will allow identification of high-risk patients at an early stage. We propose that these markers may help to guide the time of start of immunosuppressive treatment in individual patients.

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