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Original Article

Monoclonal gammopathy after intense induction immunosuppression in renal transplant patients

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Abstract

Objectives. Incidence and risk factors of post-transplant monoclonal gammopathy were studied in renal transplant patients who received their grafts between 1982 and 1992 ($n=390$ grafts). Immunoelectrophoresis was performed at annual intervals after transplantation.

Results. Forty-six cases of clonal gammopathy were detected: 35 monoclonal, 11 bi- or triclonal, with a predominance of IgG and κ light-chain subtypes (IgG, 39; IgA, 3; IgM, 4; κ, 35; λ, 19). Gammopathy was transient in 17 patients (37%). The 5-year cumulative incidence of gammopathy was 10.7%, much higher than expected for a group of similar age from the general population. Thirty of the 46 gammopathies appeared within the first 2 years of transplantation. Gammopathy never progressed to multiple myeloma during follow-up (median 1 year; (range 0–10)); one patient subsequently developed Kaposi sarcoma. The 2-year incidence of gammopathy was much higher in patients transplanted in 1989–1991 (23/142) than in 1982–1988 (7/248) ($P<0.0001$). This coincided with the use of quadruple induction immunosuppression (cyclosporin A + azathioprine + prednisone plus either ATG-Fresenius (ATG-F) or OKT3) since 1989. The risk for acquiring gammopathy within 2 years of transplantation was 14.7% (95% CI 9.2, 20.3%) in patients receiving quadruple induction therapy, but only 3.0% (CI 1.2, 6.1%) without such therapy ($P<0.0001$). The risk for patients receiving quadruple immunosuppression with OKT3 was 24.5%, significantly greater than with ATG-F (11.8%, $P<0.05$). Discriminant analysis revealed that the type of immunosuppression, but not age or year of transplantation, were independent risk factors for gammopathy.

Conclusion. Monoclonal gammopathy frequently occurs after renal transplantation. Risks are higher for patients receiving quadruple induction immunosuppression, particularly if it includes OKT3. Follow-up of these patients is warranted for the early detection of malignant transformation.

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Key words: monoclonal gammopathy; kidney transplantation; prophylactic immunosuppression; monoclonal antibodies; polyclonal antibodies

Introduction

Although monoclonal gammopathy has been frequently observed after solid-organ and bone-marrow transplantation, its incidence and spontaneous course have not been well documented. Since a significant proportion of patients with spontaneously occurring gammopathy later develop multiple myeloma or other lymphoproliferative syndromes [1], this disorder is considered a potential premalignant state. However, it is unknown whether the same holds true in the post-transplant setting.

Transplantation-associated lymphoproliferative disorders have become more common in recent years, a finding that has been related the use of more powerful immunosuppressants. Recent data from the Collaborative Transplant Study have demonstrated that patients treated with antilymphocytic substances, particularly OKT3, carry an increased risk for non-Hodgkin lymphoma [2], and similar observations have been made by others [3,4]. We became concerned when we recently witnessed a sharp increase in the incidence of monoclonal gammopathy in the kidney transplant recipients of this centre.

We therefore decided to determine the incidence and the clinical course of monoclonal gammopathy after renal transplantation and to identify potential risk factors. The Basel renal transplant population proved uniquely suitable for this purpose, because annual immunoelectrophoresis had routinely been carried out in virtually all patients since 1982.

Subjects and methods

The charts of all patients transplanted in Basel between 1.1.1982 and 31.12.1991 were reviewed. During this period, 490 transplants were performed in 451 patients, and all patients were regularly followed at our centre. During annual check-up examinations, all patients underwent determination

of standard renal and haematological parameters, a complete history and physical examination, as well as a protein- and immunoelectrophoresis. Immunoelectrophoresis was performed using a Ready-System® immunoelectrophoresis system. It was replaced by the more sensitive immunofixation from March 1992*; the latter being carried out using a Beckman® immunofixation kit using sample dilutions of 1:2 to 1:10, according to the manufacturer's specification.

We included in the present study all patients with a minimum follow-up of 1 year after transplantation. The date of last follow-up was 1 January 1994. Since the objective was to determine the incidence of *de-novo* gammopathy, patients with pre-existing gammopathy were excluded. Age and the immunosuppressive regimen were considered the two main potential risk factors.

During the time covered by the study, immunosuppression was cyclosporin A (CsA) based. Initial immunosuppression generally comprised CsA with or without prednisone (Pred) in 1982–1984, CsA + Pred + ATG-Fresenius (ATG-F) in 1985–1986, CsA + Pred + azathioprine (Aza) in 1987–1988, and CsA + Aza + Pred + either ATG-F or OKT3 (quadruple immunosuppression) from 1989 on [5]. We always attempted to reduce immunosuppression to cyclosporin A monotherapy within the first 3–5 months after transplantation unless contraindicated on clinical grounds. Rejection episodes were treated with pulse steroids and, if steroid resistant, with antilymphocytic substances (i.e. ATG-Fresenius or OKT3) from 1984–85 on.

Statistical calculations were carried out with the 'Statistica/w' software package on a personal computer (Statsoft Co., Tulsa, Oklahoma, USA). Life-table analysis was used to compute the cumulative incidence of gammopathy. The Mann-Whitney U test was used for age comparison, and the χ^2-test for the association of initial immunosuppressive regimens with monoclonal gammopathy. Discriminant analysis was used to identify risk factors contributing independently to the occurrence of monoclonal gammopathy. A P value <0.05 was considered significant.

Results

Based on the inclusion criteria, 390 of the 490 kidneys were considered suitable for analysis. Of the 100 excluded grafts, 97 did not meet the minimum requirement of having at least one immunoelectrophoresis ≥ 1 year after transplantation: 55 of these because of graft loss, 27 because of death within the first year, and 15 because the appropriate analysis was not performed or the patient was lost to follow-up. Three were excluded because of pre-existing gammopathy.

Gammopathy was detected in 46 patients. Among these, seven had received a living related donor kidney and eight a second or third cadaveric graft. The remaining 31 were first cadaveric graft recipients.

Of the 46 gammopathies, 35 were monoclonal and 11 bi- or triclonal. In most cases the amount of clonal protein detected was low and not detectable by paper protein electrophoresis. There was a predominance of IgG and κ light-chain phenotypes (Table 1).

Most of the patients developed gammopathy early after transplantation: it was detected at 1 year in 23 patients and within the first 2 years in 30 patients. The median time from transplantation to gammopathy detection was 1.5 years (range 1–8). As shown in Figure 1, the cumulative incidence appeared to progressively flatten, giving a cumulative 5-year incidence of 10.7%.

Gammopathy was transient in 17 of the 46 patients. Of the remaining 29, gammopathy persisted to the end of follow-up in 18. In 11 patients only one immunoelectrophoresis was available, and persistence could not therefore be evaluated. The follow-up after detection of gammopathy was very short (median 1 year (range 0–10)), reflecting the recent increase in *de-novo* gammopathies (see below).

In no case was progression of gammopathy to non-Hodgkin lymphoma or multiple myeloma observed. One patient with persistent serum paraprotein later developed Bence Jones proteinuria, and one patient went on to develop Kaposi sarcoma. A third patient with biclonal gammopathy was given a diagnosis of presumptive multiple myeloma because of a dense (30%) plasma cell infiltrate in the bone marrow aspirate. However, as reported elsewhere [6], all signs of disease regressed after reduction of immunosuppression, and the patient continues to do well. On 1.1.94, 40 of the 46 gammopathy patients were alive with a functioning transplant, one had lost the graft from

Table 1. Frequency and distribution of isotype and light-chain restriction

Gammopathies	46	Monoclonal	35
		Bi/triclonal	11
IgG	39		
IgA	3	Kappa	35*
IgM	4	Lambda	19*

*The sum of kappa plus lambda gammopathies exceeds 46 because of the bi-and triclonal gammopathies.

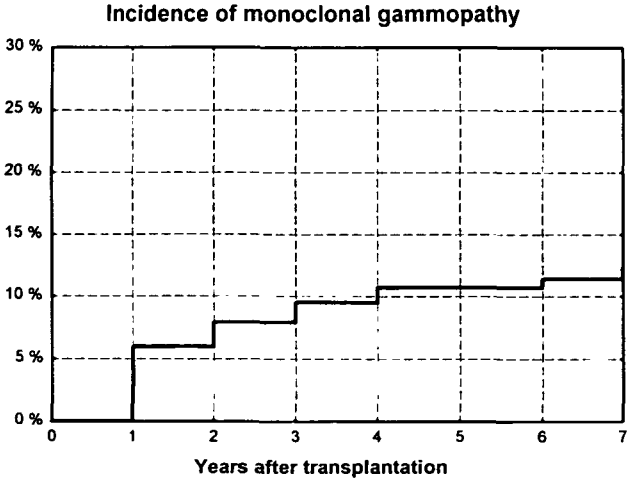

Fig. 1. Cumulative incidence of monoclonal gammopathy after renal transplantation (life table analysis).

* *Although occasional samples were screened by immunofixation after 1987, every positive sample was confirmed by immunoelectrophoresis to February 1992.*

rejection and was back on haemodialysis, and five patients had died, none of malignant disease.

The immunosuppressive regimens of the 46 patients at the time of gammopathy detection did not differ from non-gammopathy patients of the same time period (data not shown). Similarly, immunosuppression at detection of gammopathy did not differ between the 18 patients with persistent gammopathy (10 CsA monotherapy, 8 dual or triple therapy) when compared to the 17 patients with transient gammopathy (7 CsA monotherapy, 10 dual or triple therapy).

There was a remarkable increase in the incidence of *de-novo* monoclonal gammopathies since 1989 (Figure 2). When limiting the analysis to gammopathy within 2 years of transplantation ($n=30$), gammopathy was 5–6 times more common in patients transplanted in 1989–1991 (23/142) than in patients transplanted between 1982 and 1988 (7/248) ($P<0.0001$). This appeared to correlate with the initial immunosuppressive regimen used. As shown in Table 2, the risk of developing gammopathy was 3.0% for patients not receiving any antilymphocytic substance and 3.1% for patients treated with ATG-F as part of a triple induction regimen (ATG-F + CsA + Pred). However, patients receiving quadruple induction immunosuppression had a risk of 14.7% ($P<0.0001$ *versus* the other two groups). Quadruple induction with ATG-F carried a risk of 10.4%, whereas the risk was 24.0% when OKT3 was included in the regimen ($P<0.05$ OKT3 *versus* ATG-F). Approximately one-half of the gammopathy cases in each of the induction therapy groups were transient and one-half persistent; the groups were not different in this respect. The median age at transplantation of patients who developed gammopathy (52.5 years (range 16–68)) was slightly higher than of patients without gammopathy (49 years (9–70)); the difference was of borderline significance ($P=0.045$).

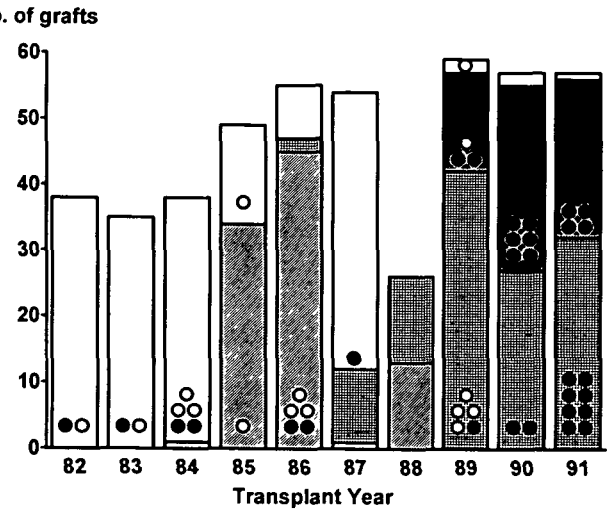

Fig. 2. Number of transplants according to different initial immunosuppression (□ IS without ATG-F or OKT3, ▨ IS with ATG-F, CsA and prednisone, ▦ ATG quadruple, ▩ OKT3 quadruple) and monoclonal gammopathies (● within 2 years of transplantation, ○ after 2 years of transplantation).

Since age, the year of transplantation, and the immunosuppressive regimen were all intercorrelated, discriminant analysis was performed to identify independent risk factors. The occurrence of gammopathy within 2 years was used as the dependent variable, the independent variables being age at transplantation, the year of transplantation and the immunosuppressive regimen: (1) no antilymphocytic substance, (2) ATG-F induction with CsA-Pred, (3) ATG-F quadruple induction, or (4) OKT3 quadruple induction). Only the immunosuppressive regimen ($P<10^{-5}$), but not age or the year of transplantation proved to be an independent predictor. Thus, the increase of early gammopathies in recent years was associated with the use of quadruple immunosuppression, particularly if the latter included OKT3.

To eliminate the potential bias caused by the introduction of immunofixation in March 1992, the same analysis was carried out in a subset of patients transplanted before November 1990 (333 patients, 15 gammopathies, all diagnosed by immunoelectrophoresis). Discriminant analysis again revealed only the immunosuppressive regimen ($P<0.02$), but not age or year of transplantation as independent predictors. Of the 15 gammopathies, three had received ATG-F quadruple induction and five had received OKT3 quadruple induction, giving risks of 5.0% and 19.2% for the two groups ($P\approx0.09$). Again, the gammopathy risk with quadruple induction immunosuppression was significantly greater than without (9.3% *versus* 2.8%, $P<0.05$).

Discussion

The 10.7% incidence of monoclonal gammopathy at 5 years found in the present study makes gammopathy

Table 2. Gammopathies within 2 years of transplantation. Absolute risk according to immunosuppressive regimen

Initial immunosuppression	MG	no MG	Risk	95% confidence interval
No induction	5	164	3.0%	1.0–6.7
ATG triple induction	2	63	3.1%	0.4–10.7
Quadruple induction	23	133	14.7%	9.2–20.3
Quadruple ATG-F	11	95	10.4%	4.6–16.2
Quadruple OKT3	12	38	24.0%	13.1–38.2

MG = monoclonal gammopathy, ATG-triple = ATG-F + CsA + Pred, Quadruple ATG-F = ATG-F + CsA + Aza + Pred, Quadruple OKT3 = OKT3 + CyA + Aza + Pred.

a common disorder after renal transplantation. There is no doubt that this is much more than the prevalence of monoclonal gammopathy in non-transplanted age-matched controls. Data from the general population show a 1% incidence of clonal gammopathy in cohorts over 50 years of age, which increases to 3% in persons over 70 [1,7]. The highest prevalence figures are reported in the octogenarian nursing-home population [8]. This entity, now termed 'monoclonal gammopathy of unknown significance' (MGUS), is clearly a premalignant condition of the elderly, and half of those surviving long enough transform to a malignant state, usually multiple myeloma, after a median follow-up of 19 years [1].

In contrast, the outcome of transplant-associated gammopathy is still unclear. One carefully conducted study with 3-month follow-up intervals reported a 23% incidence of gammopathy within $2\frac{1}{2}$ years after renal transplantation, 80% of which were transient [9]. Gammopathy was more common in patients with chronic rejection, and therefore with more intense immunosuppression. A cross-sectional study reported a 30% prevalence of gammopathy in renal transplant patients [10]. Again, gammopathy was transient in 17 of 23 patients who were further followed, but two patients developed myeloma. Age, but not the duration of immunosuppression, appeared to favour gammopathy occurrence. A third study, which reported a 12% incidence at 8 years but with 85% appearing within 6 months of transplantation, reported age and the type of immunosuppression to be risk factors: patients treated with CsA + prednisone were at higher risk than patients on Aza + prednisone or CsA + Aza + prednisone [11]. Two patients developed multiple myeloma and one a solitary plasmacytoma. Of note is the fact that the myelomas appeared 10 and 13 years after transplantation. Lastly, in a cross-sectional study of 110 renal transplant patients, monoclonal gammopathy was detected in four, one of whom suffered from myeloma [12].

Consistent with these reports, the course of gammopathy in the present study was very variable: some patients had transient gammopathy, while some maintained a steady, low level of paraprotein production. Although no lymphoma or myeloma was found in the 46 patients of the present study, this should not lead one to neglect the follow-up of these patients. One of the patients developed Kaposi sarcoma, indicative of a profound state of immunosuppression, and plasma-cell proliferation in the bone marrow of an additional patient was so pronounced that myeloma was strongly suspected. Gammopathy was the first sign of Non-Hodgkin lymphoma in a third patient, who was transplanted in 1981, just before the beginning of the present study.

The recent bout of clonal gammopathy at our centre (Figure 2) prompted us to evaluate the role of induction immunosuppression. By limiting the analysis to patients with *de-novo* gammopathy within 2 years of transplantation, we eliminated the bias of the longer observation period in patients transplanted before 1989. The present analysis strongly suggests that the quadruple immunosuppressive protocol used since 1989, which included CsA, azathioprine, steroids, and either ATG-F or OKT3, favoured the development of gammopathy. It remains open to debate whether the critical factor is the overall intensity of immunosuppression or the addition of antilymphocytic globulins (in particular, OKT3). Favouring the former possibility is the fact that triple induction immunosuppression with ATG-F, CsA and steroids did not appear to promote gammopathy (Table 2).

The change in the screening method for gammopathy from immunoelectrophoresis to immunofixation in March 1992 may be suspected to cause a methodological bias. However, when the statistical analysis was applied to a data subset where only immunoelectrophoresis was considered, results very similar to those in the entire set were obtained. The only exception was that due to the smaller patient number, the gammopathy incidence between the ATG and OKT3 quadruple groups no longer differed significantly. We therefore considered it permissible to analyse the immunoelectrophoresis and immunofixation data together.

Gammopathy therefore appears to occur in circumstances similar to post-transplantation lymphoma. A recent analysis of data from the Collaborative Transplant Study demonstrated an increased risk for non-Hodgkin lymphoma with CsA/azathioprine dual therapy and a further increase with the use of antilymphocytic globulins, particularly OKT3 [2]. Like the gammopathies of the present study (Figure 1), most of these lymphomas appeared early after transplantation. This may be a hint that post-transplantation lymphoma and gammopathy have some similarities in their pathogenesis.

The mechanisms leading to post-transplantation gammopathy, however, are essentially speculative. One hypothesis would propose that diminution of T-cell mediated immune surveillance might temporarily allow B-cell proliferation to escape, particularly if promoted by viral infection. As a rule, control would later be regained, resulting in stabilization or regression of the expanded clones. However, if control remained suppressed for a prolonged time period, progression to lymphoma or myeloma might ensue. Alternatively, in children with severe combined immunodeficiency undergoing bone marrow transplantation, a particularly high incidence of transient gammopathies has been observed, leading to the hypothesis that recovery of the immune system may be asynchronous [13,14]. Clones which recover faster than others then can cause gammopathy, even though the overall system remains controlled.

It should be emphasized that follow-up in the present study is far too short to establish the dignity of this syndrome. As illustrated by the literature cases [10], transformation from gammopathy to myeloma usually occurs late; it may take over a decade to develop. Thus, the diagnosis of post-transplant gammopathy has two potentially dangerous implications: early after transplantation it may herald lymphoma and many

years later, there may be transformation to multiple myeloma.

In summary, monoclonal gammopathy occurs commonly after renal transplantation. Although it is frequently transient, physicians should be aware that this diagnosis carries an increased risk for lymphoma early after transplantation and for myeloma many years later. The present study demonstrates for the first time an association of post-transplant monoclonal gammopathy with immunosuppressive induction therapy, namely a quadruple regimen which includes antilymphocytic globulins.

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