Membranous nephropathy and cancer: Epidemiologic evidence and determinants of high-risk cancer association

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The association between membranous nephropathy (MN) and cancer is often mentioned in textbooks but poorly substantiated, and the characteristics of cancer-associated MN are unknown. To address these questions, we studied a cohort of 240 patients with MN, among them 24 had malignancy at the time of renal biopsy or within a year thereafter. The incidence of cancer was significantly higher in these patients than in the general population (standardized incidence ratio 9.8 [5.5–16.2] for men and 12.3 [4.5–26.9] for women). The frequency of malignancy increased with age. At the time of diagnosis, clinical presentation did not differ between the patients with cancer-associated MN and those with idiopathic MN, but smoking was more frequent among patients with cancer. Analysis of renal biopsies revealed that the number of inflammatory cells infiltrating the glomeruli was significantly higher in patients with cancer-associated MN ($P = 0.001$). The best cutoff value for distinguishing malignancy-related cases from controls was eight cells per glomerulus. Using this threshold led to a diagnosis of cancer-associated MN with a specificity of 75% and a sensitivity of 92%. In patients with cancer-associated MN, there was a strong relationship between reduction of proteinuria and clinical remission of cancer ($P < 0.001$). In conclusion, our study provides epidemiologic evidence of an excess of cancer risk in patients with MN. It also shows that age, smoking, and the presence of glomerular leukocytic infiltrates strongly increase the likelihood of malignancy in MN patients.

Kidney International (2006) **70,** 1510–1517. doi:10.1038/sj.ki.5001790; published online 30 August 2006

KEYWORDS: membranous nephropathy; nephrotic syndrome; cancer

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Received 24 March 2006; revised 28 May 2006; accepted 20 June 2006; published online 30 August 2006

Membranous nephropathy (MN) is the most common cause of adult-onset nephrotic syndrome in Caucasian patients. It is characterized by the accumulation of immune deposits on the epimembranous aspect of the glomerular basement membrane in the absence of significant intraglomerular cellular proliferation. There is convincing evidence that MN can be associated with various disease processes, including infections, autoimmune diseases, and drug toxicity.[1] Although the association between MN and cancer is frequently reported,[1–10] it has been pointed out that the notion of paraneoplasic MN is based only on a limited number of old series and case reports showing a temporal link between the course of MN and tumor activity, as reviewed by Glassock[2] and Ronco.[3] For example, the frequency of cancer in the MN population has been variously estimated at between 5 and 22%,[4–8] carcinomas being the tumors most frequently associated with MN. Similarly, as pointed out by Ronco[3] only isolated cases of remission of MN after treatment have been reported.[3,10–18] In addition, up to now the available data have not permitted comparison of the incidence of cancer in the MN population with that in the general population, nor the determination of distinguishing clinical, laboratory, or morphological features which should lead physicians to suspect a cancer-associated MN. To try to resolve these issues, we have analyzed the incidence of cancer in a cohort of 240 patients with MN, as well as the characteristics and outcome of these cancer-associated MNs.

RESULTS

Incidence of cancer among patients with MN

The prevalence of cancer among patients with MN was 10%. It significantly increased with age ($P < 0.001$), but was not related to gender (Table 1).

Among the 21 incident cases of cancer associated with MN, the tumor was symptomatic at the time of renal biopsy in only 11 patients (52%). Nine presented specific tumor-related symptoms (cough, urinary retention, …) and two had impairment of general health status. For the remaining 10 patients (48%) the tumor was asymptomatic and only

Table 1 | Distribution of cancer cases among patients with MN by age and gender

	Men			Women			Total		
Age	MN	T	%	MN	T	%	MN	T	%
18–54	88	2	2.3	47	1	2.1	135	3	2.2
55–64	21	2	9.5	11	1	9.1	32	3	9.4
⩾65	45	13	28.9	28	5	17.9	73	18	24.7
Total	154	17	11.0	86	7	8.1	240	24	10.0

MN, membranous nephropathy.
MN, number of patients with MN; T, number of patients with MN and cancer; %, percentage of MN patients with cancer.

Table 2 | SIR of cancer in a cohort of 240 patients with MN, as compared to the French general population

	Men			Women		
Age	O	E	SIR (95 %CI)	O	E	SIR (95 %CI)
18–54	2	0.20	10.2 (1.1–36.7)	1	0.10	9.5 (0.1–52.7)
55–64	2	0.23	8.6 (1.0–31.1)	1	0.08	13.0 (0.2–72.4)
⩾65	11	1.10	10.0 (5.0–17.9)	4	0.30	13.2 (3.5–33.7)
Total	15	1.53	9.8 (5.5–16.2)	6	0.49	12.3 (4.5–26.9)

CI, confidence interval; MN, membranous nephropathy; O, number of incident cancers observed in the group of patients with MN; E, expected number of cancers in this group of patients, estimated using 2000 cancer incidence rates in the French population.[35] ; SIR, standardized incidence ratio.

recognized by systematic diagnostic procedures triggered by the diagnosis of MN.

Compared with the general population, the incidence of cancer among patients with MN was about 10 times higher in all age groups and in both genders (Table 2). Standardized incidence ratio (SIR) remained highly significant in both men and women, even if it was assumed that none of the 47 patients with no medical record or lost to follow-up developed cancer (SIR 8.6 [95% confidence interval (CI): 4.8–14.2], and 10.7 [95% CI: 3.9–23.4], respectively). After restricting the analysis to patients whose tumors were symptomatic at the time of diagnosis of MN, SIR also remains significant in men: SIR 7.1 [95% CI: 3.4–13] for men and 4.4 [95% CI: 0.5–16] for women.

Types of cancer associated with MN

The vast majority of tumors associated with MN were carcinomas (20 cases, 83.3%), and the most frequent localizations were lung (eight cases) and prostate (five cases), (Table 3). In men, the SIR was 31.9 for lung cancer (95% CI: 12.8–65.7) and 12.1 for prostate cancer (95% CI: 4–28.3). Two tumors were of hematopoietic origin and two others were rare malignancies: a mediastinal liposarcoma and a malignant micronodular thymoma with capsular and perithymic adipose tissue invasion.

Baseline characteristics of cancer-associated membranous nephropathies

Patients with cancer-associated MN were significantly older than controls, but the male to female sex ratio was similar between the two groups (Table 4). Clinical and laboratory data at the time of renal biopsy did not significantly differ between cases and controls, with the exception of estimated glomerular filtration rate (Table 4). However, this difference was no longer significant after adjusting for age. The higher percentage of hematuria in cases than in controls was borderline significant ($P=0.06$). Patients with cancer-associated MN were more often heavy smokers (⩾20 pack-years) than controls ($P<0.01$).

A thrombotic event was diagnosed during the initial evaluation of the nephropathy in 25% of patients with cancer-associated MN, and it was responsible for pulmonary embolism in one case. No renal vein thrombosis was observed, in spite of systematic search by colored-coded duplex sonography.

Comparison of renal biopsies between patients with cancer-associated MN and the 24 matched controls are shown in Table 5. Patients with cancer-associated MN tended to have more often stage I MN, but this difference was not statistically significant. More importantly, the number of inflammatory cells infiltrating the glomeruli was significantly higher in the group of patients with cancer-associated MN ($P=0.001$), (Table 5 and Figure 1). According to the receiver-operating characteristic curve, the best cutoff value for distinguishing malignancy-related cases from the controls was eight cells per glomerulus. Using this threshold led to the diagnosis of cancer-associated MN with a specificity of 75% and a sensitivity of 92%. The area under the curve was 0.92. The number of inflammatory cells per glomerulus was not correlated with age, history of smoking, stage of MN, type of tumor, or the presence of metastases.

Course of patients with cancer-associated MN

Dialysis-free survival was not used as an end-point, as dialysis may not have been offered to all patients with cancer. As expected, survival with estimated glomerular filtration rate

Table 3 | Types of cancer and outcome of tumor and nephrotic proteinuria among 24 patients with cancer-associated MN

Localization of tumor	Histology	N	M/F	Stage	Treatment of tumor	Remission	
						Tumor	Nephrotic-range proteinuria
C34 Bronchus and lung	Adenocarcinoma	4	3/1	T2N1M0	S	+	C
				T2N2M0	S+R	–	–
				T2N2M1	R	–	–
				T2N2M1	—	–	–
	Squamous cell carcinoma	4	4/0	T1N0M0	S	+	C
				T2N1M0	S+R+Ch	+	C
				T2N2M0	S+R	+	P
				T2N2M0	S+R	–	–
C61 Prostate	Adenocarcinoma	5	5/0	T2NxM0	H	+	C
				T2NxM0	H+Cs+R	+	P
				T3NxM0	H+R	–	–
				T3NxM0	S+H+Cs	–	–
				T3NxM1	H	–	–
C16 Stomach	Adenocarcinoma	2	1/1	T1N0M0	S	+	–
				T2N1M1	—	–	–
C18 Colon	Adenocarcinoma	1	1/0	Dukes D	Ch	–	–
C32 Larynx	Squamous cell carcinoma	1	1/0	T2N2M0	R	–	–
C37 Thymus	Micronodular thymoma	1	0/1	Masoaka II	S+R	+	C
C38 Mediastinum	Liposarcoma	1	1/0	T3N0M0	S	+	P
C67 Bladder	Transitional cell carcinoma	1	1/0	T4N1M0	S+R	–	–
C53 Cervix uteri	Squamous cell carcinoma	1	0/1	T2N0M0	R	+	–
C50 Breast	Infiltrating duct carcinoma	1	0/1	T2N0M0	S+R+H	+	–
C83 Diffuse non-Hodgkin's lymphoma	Follicular B-cell lymphoma	1	0/1		Cs+Cy	+	C
C92 Chronic myeloid leukaemia		1	0/1		Ch	–	–

Numbers heading the cancer localization are from the International Classification of Disease (ICD-10-CM); C, complete remission of nephrotic-range of proteinuria; Ch, chemotherapy without steroids or alkylating agents; Cs, chemotherapy including steroids; Cy, chemotherapy including alkylating agents; H, hormonal therapy M/F, male-to-female ratio; MN, membranous nephropathy; *N*, number of patients; P, partial remission of nephrotic-range proteinuria; R, radiotherapy; S, surgery.

Table 4 | Clinical and laboratory data at the time of renal biopsy for patients with cancer-associated MN and controls

Characteristics	Cancer-associated MN	Controls	Age-adjusted *P*-value
N	24	129	—
Men	71%	71%	NS
Age (years)	73 (65–78)	52 (39–69)	<0.001
Age $\geqslant$65 years	75%	33%	<0.001
Proteinuria (g/day or g/g creatinine)	6.8 (5.0–10.3)	6.0 (3.6–8.1)	NS
Proteinuria >3 g/day or g/g creatinine	96%	84%	NS
Proteinuria >10 g/day or g/g creatinine	25%	17%	NS
Serum albumin (g/l)	20 (17–25)	23 (17–28)	NS
Hematuria	54%	34%	NS
Serum creatinine (μmol/l)	109 (84–156)	88 (73–107)	NS
eGFR (mL/min/1.73 m^2)	59 (40–83)	78 (61–95)	NS
eGFR <60 ml/min/1.73 m^2	54%	25%	NS
eGFR <30 ml/min/1.73 m^2	17%	7%	NS
Hypertension	71%	60%	NS
SBP (mmHg)	140 (125–158)	140 (130–160)	NS
DBP (mmHg)	80 (70–88)	80 (72–90)	NS
Ever smoking	68%	56%	NS
Heavy smoking ($\geqslant$20 pack-years)	68%	29%	<0.01

DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate; MN, membranous nephropathy; NS, nonsignificant; SBP, systolic blood pressure.
Results are expressed as median (interquartile range) or as percentage.
NS: *P*-value >0.05.

>15 ml/min/1.73 m^2 was significantly worse in patients with cancer-associated MN than in those with idiopathic MN (Figure 2). Sixteen patients (66.7%) with cancer-associated MN died during follow-up, as compared to only two patients (8.3%) with idiopathic MN ($P<0.001$). The death was secondary to neoplasia in seven patients (43.8%), to complications of nephropathy in three patients (two infections, one pulmonary embolism), to cardiovascular disease in three patients and to various causes in the last three. The two patients with idiopathic MN died from sepsis and vascular

Table 5 | Comparison of renal biopsies between patients with cancer-associated MN and controls

Histological parameter	Cancer-associated MN	Controls	*P*-value
Stage I MN	33.3%	12.5%	NS
Stage II MN	41.7%	58.3%	NS
Stage III MN	25%	29.2%	NS
Sclerotic glomeruli > 25%	16.7%	25%	NS
FSGS lesions[a]	12.5%	29.2%	NS
Glomerular congestion	20.8%	33.3%	NS
Number of inflammatory cells/glomerulus[b]	12.8 (10.2–15.5)	5 (3.5–7)	0.001
> 8 Inflammatoty cells/glomerulus	91.7%	25%	< 0.001
Interstitial fibrosis > 10%	58.3%	45.8%	NS
Interstitial fibrosis > 20%	29.2%	45.8%	NS
Arteriosclerosis	70.8%	83.3%	NS
Glomerular microthrombi	8.3%	0	NS
Focal cortical atrophy	4.2%	0	NS

FSGS, focal segmental glomerulosclerosis; MN, membranous nephropathy; NS, nonsignificant.
Results are expressed as median (interquartile range) or as percentage.
[a]Superimposed lesions of FSGS.
[b]Includes polymorphonuclear and mononuclear leukocytes.

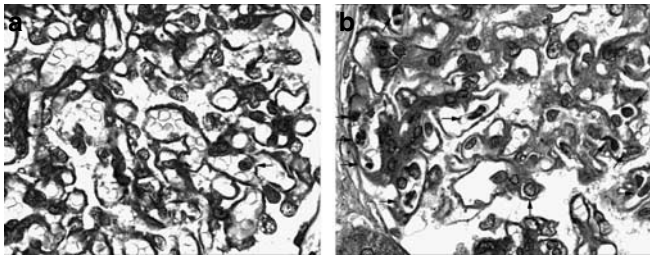

Figure 1 | Representative example of a glomerulus from a patient with idiopathic MN (a) and from a patient with cancer-associated MN (b). (**a**) The glomerulus contains only one mononuclear cell (→). (**b**) Numerous inflammatory cells are present within the glomerulus (→). Masson's trichrome stain, original magnification × 100.

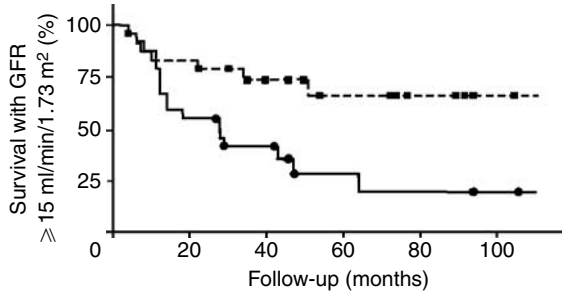

Figure 2 | Kaplan–Meier plot of survival with glomerular filtration rate ⩾ 15 ml/min/1.73 m^2 in patients with cancer-associated MN and controls. Solid line: patients with cancer-associated MN. Dotted line: patients with idiopathic MN. Dot: censored patient.

stroke. The median survival time after the diagnosis of MN was only 13 months (interquartile range 11–36 months) in patients with cancer. Ten patients (41.7%) with cancer-associated MN developed chronic kidney disease stage 5 versus 8 (33.3%) with idiopathic MN (NS).

Twenty-three patients with cancer-associated MN and 20 controls had nephrotic-range proteinuria at the time of diagnosis. During follow-up, complete remission was observed in six cases and six controls and partial remission in three cases and seven controls. In the malignancy-related group, all remissions occurred in patients whose tumor was also in remission, whereas tumor was considered as being in remission, in only three of the 14 patients with persistent nephrotic-range proteinuria. Thus, there is a significant relationship between the reduction in proteinuria and remission of cancer ($P < 0.001$). The treatments received by the 24 patients with cancer-associated MN are summarized in Table 3. Only two of the nine patients with combined remission of both nephrotic syndrome and cancer were treated with chemotherapy including corticosteroids (both patients) and cyclophosphamide (one patient). The other patients were treated by surgery (6/9), radiotherapy (4/9) and/or hormonal therapy (1/9), or chemotherapy not using steroids or alkylating agents (1/9).

DISCUSSION

As with earlier studies, we observed a high frequency of cancer in patients with MN. However, ours is the first to show a significant increase in cancer among patients with MN in comparison with the general population. It is also the first to suggest that there is a histological marker predictive of cancer, and to indicate a clear link between decrease in proteinuria and tumor remission. These findings are noteworthy because they are based on a large and nonselected cohort of patients with histologically proven MN.

Our study shows that 10% of patients diagnosed with MN had cancer. These data are in agreement with older series in which the frequency of cancer in MN population has been variously estimated from 5 to 22%.[4–8] However, none of these series has compared the incidence of cancer in this group with that in the general population. In our study, this was about 10-fold higher in patients with MN than in the general population. Even when restricting the analysis to the patients whose tumors were symptomatic at the time of diagnosis of MN, the worst case assumption in a country with widespread cancer screening, the incidence remained high. Thus, the excess of malignancy in patients with MN cannot be explained by a detection bias. Although the observed-to-expected

ratios were similar in all age groups, cancer was diagnosed in one out of four patients with MN over age 65 and only in one out of 50 patients under age 55 (Table 2). In contrast, gender was not a determinant of association with cancer among MN patients. To present, the sole population study has been that of Birkeland and Storm, which found an excess of cancers in patients with glomerulopathies, including MN in men.[9] In that study the SIR was 1.79 for men (95% CI: 0.95–3.05) and 1.97 for women (95% CI: 0.79–4.07). The differences in SIR between our study and the one of Birkeland and Storm may be due to methodological differences. In the latter study, classification of MN as being cancer-associated was solely based on a record linkage between the Danish Kidney Biopsy and Cancer Registries, without systematic analysis of individual medical records. This method may have lacked sensitivity in detecting cancer cases, and led to an underestimation of SIR.

Our study establishes a clear correlation between remission of cancer and remission of nephrotic syndrome in patients with cancer-associated MN. Up to now, only isolated cases of remission of MN after treatment of the neoplasm have been reported, with a total of less than 20 cases related to tumors in the last 10 years.[3,10–18] In our study, complete remission of nephrotic syndrome occurred in six out of 12 patients whose tumor was in remission but in none of 12 patients whose tumor was not in remission. Importantly, only one patient with complete remission of nephrotic syndrome received a chemotherapy including steroids plus alkylating agents. The temporal link between complete remission of nephrotic syndrome and tumor remission strongly argues in favor of a causal relationship between the two diseases.

As suggested by previous case reports, carcinomas were by far the tumors most often associated with MN. In our cohort, the cancers most frequently associated with MN were lung (eight) and prostate (five), whereas association between prostate cancer and MN has rarely been reported.[19] It is likely that prostate cancer has been under-recognized in older series owing to the lack of serologic marker, whereas measurement of prostate-specific antigen is now systematic in aging men. Based on our study showing an increased incidence of prostatic and lung cancer among MN patients, we suggest systematic investigation for possible prostatic or pulmonary tumor among patients over 65 years of age, as the first priority, before investigation of other possible primary sites. Interestingly, none of the malignancies associated with MN were skin cancer, despite the fact that this type of cancer is extremely common in elderly people. This is consistent with the fact first that skin cancers associated with MN have rarely been reported in the literature, and second that only melanoma, Kaposi's sarcoma or cutaneous T-cell lymphomas have been reported in association with MN.[20–22]

In our study, the clinical presentation of cancer-associated MN could not be distinguished from that of idiopathic MN, with one noticeable exception; heavy smoking ($\geqslant$20 pack-years) was more frequent among patients with cancer. In previous studies of biopsies in malignancy-related MN, efforts have focused on detection of tumor antigens, rather than on comparative studies of potential differences separating such cases from idiopathic MN. In the present study, systematic analysis revealed that on average, the number of inflammatory cells infiltrating the glomeruli was more than twofold higher in patients with cancer. Using a receiver-operating characteristic curve, we could determine a cutoff value of eight cells per glomerulus. The area under the curves indicates that based on this marker, 92% of the patients would be correctly classified as having or not having cancer. Thus, the determination of the number of leukocytes infiltrating the glomerular capillaries appears to be a useful exercise, and if they are increased, an additional incitement to pursue the investigation of malignancy in these MN patients. However, although the basic observation of an increase in the number of glomerular leukocytes is clearly valid given the sample sizes (272 glomeruli in cancer-associated MN and 311 glomeruli, in idiopathic MN), we recognize that the cutoff value that we have defined needs to be validated in an independent cohort.

It is not clear why patients with cancer-associated MN have more inflammatory cells infiltrating the glomeruli. One possibility would be that the deposition of immune complex containing tumor-associated antigens induces the release of proinflammatory molecules. However, one should keep in mind that the number of infiltrating cells is limited, much smaller than what is seen in renal biopsies from patients with mixed cryoglobluliemia or post-infectious glomerulonephritis. Numerous studies have attempted to establish pathophysiologic links between the tumor and the renal lesion. Different tumoral antigens[23–25] or their specific antibodies[26,27] have been identified in the kidneys of these patients, but without definite proof of their pathogenic nature. Interestingly, Ohtani *et al.*[28] found that the staining observed with anti-immunoglobulin (Ig)G1 and anti-IgG2 antibodies was stronger in cancer-associated MN than in idiopathic MN. These results suggest that the immune processes involved in the pathogenesis of cancer-associated MN are different from those of idiopathic MN, with a predominance of Th1-type responses in cancer-associated MN.[28]

Although this study is the largest cohort of cancer-associated MN to date, the number of cases remains small and we may have lacked power in some comparisons with idiopathic MN. A second limitation is that this is strictly an observational study, and it seems likely that more systematic search for cancer might have revealed that the incidence of cancer among MN patients was still underestimated. However, as we have shown, a wide variety of cancers were associated with MN, such that, with the exception of lung and prostate primaries, a systematic strategy for investigation of MN patients cannot be clearly defined.

This study has important clinical implications. First of all, it confirms and gives dimensions to the long-held belief that there is an increased incidence of cancer, principally carcinomas, among MN patients, particularly in those over

age 65. Secondly, it offers a morphologic screening tool, in the form of glomerular leukocytes on renal biopsy. Their presence in numbers is indicative of a particularly high risk of cancer in these patients. This is particularly useful, given that in more than 50% of patients the tumor was asymptomatic at the time of renal biopsy. The study also points to two particularly frequent primary sites, lung and prostate, which must be systematically sought in patients over age 65. Beyond these two possibilities, the clinical workup should be guided by the clinical situation and risk factors (e.g. smoking as a risk factor in lung, upper respiratory, and bladder primaries).

In conclusion, our cohort strongly supports the existence of an association between cancer and MN. It also points out two risk factors, age and smoking, and suggests a new morphological risk marker. Further research is needed to define the best cancer screening strategy in MN patients.

MATERIALS AND METHODS

Subjects

This study has been conducted in 11 Departments of Nephrology located in the Paris region (see Appendix for a list of participating centers), after being approved by the ethics committee of Paris-Saint Louis. Patients were identified on the basis of the renal biopsy files of the affiliated Department of Pathology. All adult patients (>18 years old) in whom a MN was first diagnosed between January 1994 and June 2001 were considered for inclusion. Medical records were available for 240 (84%) out of 287 patients on file; data systematically included antinuclear antibodies, serology for hepatitis B and C, current treatment and whether cancer was diagnosed before, at the time of, or following the diagnosis of MN. All these records were reviewed by two nephrologists (X Belenfant and O Kourilsky) and one pathologist (Y Allory), in order to confirm the diagnosis of MN and determine its etiology. MN was considered as being idiopathic in 141 cases, and associated with autoimmune disease, infection or drug toxicity in 75 cases, and with cancer in 24 patients. In all 24 cases, malignancy was histologically proven. The diagnosis of cancer and MN was performed within a year in 21 cases, and these patients will be further referred to as incident cancer cases. In the three remaining patients, cancer was known more than 1 year before MN (13, 16, and 60 months, respectively), but was still active at the time of renal biopsy. These patients were therefore considered in the group of cancer-associated MN.

Controls included all patients with idiopathic MN. Of these, 24 patients were randomly selected for studying renal biopsies and long-term outcome and were considered as matched controls. Randomization was performed after stratifying by age (<55, 55–64, and $\geqslant 65$ years) and gender in order to obtain the same number of cases and controls in each stratum.

Clinical and biological data

Baseline data at the time of renal biopsy were obtained from medical records for all cases and 129 (92%) of the controls with idiopathic MN, including: age, sex, ethnicity, comorbidities, past medical history, past and current medications, blood pressure, serum creatinine, serum albumin, hemoglobin, proteinuria (expressed in g/day or as a protein to creatinine ratio), urine sediment, and smoking (ever versus never, number of pack-years). In cases and matched controls, these data were also gathered up to June 2004.

Hypertension was defined by a systolic blood pressure >140 mmHg or a diastolic blood pressure >90 mmHg or the need for anti-hypertensive medication. Nephrotic range proteinuria was defined by proteinuria >3 g/day or a protein to creatinine ratio >3 g/g. Hematuria was defined by the presence of more than 10 red blood cell per mm^3. Glomerular filtration rate was estimated using the abbreviated Modification of Diet in Renal Disease Study equation,[29] and chronic kidney disease was classified according to Kidney Disease: Improving Global Outcomes (KDIGO) guidelines.[30] Complete remission of nephrotic range proteinuria was defined by proteinuria <0.3 g/day or protein to creatinine ratio <0.3 g/g. Partial remission was defined by proteinuria <3 g/day and a 50% reduction in proteinuria.

For patients with cancer, the associated tumor was classified according to the International Classification of Diseases ((ICD)-10-CM of World Health Organization (WHO)),[31] and staged according to the international tumor node metastasis classification.[32]

Analysis of renal biopsies

Renal biopsies from all patients with cancer-associated MN and from the matched controls were reviewed by two renal pathologists (DN and GSH), blinded to the clinical and laboratory data. They all contained at least nine glomeruli. They were stained by standard methods for light microscopy analyses. Immunofluorescence studies were performed using polyclonal antibodies to IgA, IgG, IgM, fibrin, albumin, C3, C4, and C1q (Behringwerke, Marburg, Germany) and anti-kappa and anti-lambda light chains monoclonal antibodies (Dako, Glostrup, Denmark).

MN were classified into four stages according to the criteria of Bariéty and Ehreinreich: stage I, normal basement membrane on light microscopy associated with evidence of IgG deposits on immunohistochemical studies; stage II, presence of basement-membrane spikes; stage III, incorporation of some deposits into the basement membrane; and stage IV, markedly thickened basement membrane.[33]

A variety of basic lesions were systematically evaluated on each renal biopsy. Sclerotic glomeruli with evidence of focal segmental glomerulosclerosis were counted and expressed as percentages. The degree of interstitial fibrosis was estimated as a percentage of the parenchyma. Other lesions, including arterial, arteriolar, tubular lesions, and inflammatory infiltrates, were evaluated on a semi-quantitative scale of 0–3. The presence of intraglomerular thrombi was also evaluated.

The initial semiquantitative analyses showed more glomerular inflammatory infiltrates in cancer-associated MN than in idiopathic MN. Therefore, for each biopsy, all leukocytes, both polymorphonuclear and mononuclear, found within the capillary lumens of all glomeruli, were systematically counted. For each glomerulus, we considered the section with the largest diameter. The mean number of inflammatory cells per glomerulus was then calculated for each biopsy. A total of 583 glomeruli were analyzed (272 in the group of patients with cancer and 311 in the control group). The interobserver variability of the count was 10.8%.

Statistical analyses

SIR of cancer in the cohort of patients with MN as compared to the general population was estimated using 2000 cancer incidence rates in France as the reference.[34] Observed cases were the 21 incident cancer cases. Expected cases were calculated for each 5 years stratum of the cohort and by gender. National estimates of overall and site-specific cancer incidence by gender and 5 year age group were

provided by the National Institute of Public Health Surveillance (InVS) cancer registry network[35] which methods are described in.[34] SIR was estimated by age group and by gender as well as for the most frequent types of cancers (lung and prostate). To assess the robustness of these estimates, we performed two subsidiary analyses. First, in order to limit the potential overestimation of SIR that may have resulted from the screening for malignancies in asymptomatic patients with MN, we performed an analysis including only patients whose tumor was symptomatic at the time of diagnosis of MN. Second, to assess the magnitude of the error which may have resulted from not including the 47 patients whom medical record could not be traced, SIR were estimated assuming that none of them had developed cancer. For these subjects, we used date of birth and gender available from the renal biopsy files.

Patient's baseline clinical and biological data were compared between cases and the entire control group using the logistic regression adjusting for age. Renal biopsy and follow-up data were compared between cases and the 24 matched controls. To study the usefulness of the number of inflammatory cells per glomerulus as a specific marker of cancer in patients with MN, a receiver-operating characteristic curve was plotted to estimate the best cutoff in terms of making the highest sensitivity + specificity/2. We also determined the area under the curve to evaluate the ability of this marker to discriminate patients who may or may not have cancer.

Kaplan–Meier survival estimates were calculated for the death or chronic kidney disease stage 5 (dialysis or estimated glomerular filtration rate $<15\,ml/min/1.73\,m^2$) whichever came first. Survival curves were compared between cancer-associated MN cases and controls using the log-rank test.

ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Health (PHRC AOM00022 to JR, and Appel d'Offres Environnement-Santé to BS) and Ministry of Research and Technology (Appel d'Offres Cohortes Collections 2000 to BS).

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APPENDIX: GN-PROGRESS Study Group

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