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Invasive Aspergillosis after Kidney Transplantation: Case-Control Study

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40 words summary: This study reveals renal transplantation-specific risk factors for invasive aspergillosis: leukopenia and duration of renal replacement therapy for early infection, CMV seropositivity of the donor for late infection. Leukopenia, disseminated infection and serum galactomannan index correlate with mortality.

ABSTRACT

Background: Transplant recipients are at risk for invasive aspergillosis (IA), associated with a significant mortality rate. Renal transplantation-specific risk factors have not been established.

Methods: Forty-one adult kidney transplant recipients diagnosed with proven or probable IA from 1995 through 2013 were identified by search of the computerized patient files in the University Hospitals Leuven. The control population in this 1:2 case control study consisted of the two patients who received a kidney transplant immediately before and after each identified patient and did not develop IA (n = 82).

Results: Leukopenia after kidney transplantation increased the risk of IA among all patients (OR, 2.345; 95% CI, 1.084-5.071). For early-onset infection (i.e., occurred during the first 3 months after transplantation), a longer duration of renal replacement therapy pre-transplant and the occurrence of leukopenia were risk factors (OR per year, 1.192; 95% CI, 1.006-1.413 and OR, 3.346; 95% CI, 1.063-10.527, respectively), while donor CMV seropositivity increased the risk for late-onset IA (i.e., occurred >3 months after transplantation) (OR, 3.677; 95% CI, 1.388-9.743). Twelve-week mortality rate was 39%. Disseminated infection, leukopenia and the height of the serum galactomannan index were associated with an increased risk of death (HR, 5.080; 95% CI, 1.740-14.830 and HR, 3.198; 95% CI, 1.183-8.649 and HR, 1.371; 95% CI, 1.123-1.674, respectively).

Conclusions: Prolonged renal replacement therapy before kidney transplantation increases the risk of IA early after transplantation. The height of the serum galactomannan index predicts mortality.

INTRODUCTION

Although *Aspergillus* spores are ubiquitous in nature, invasive infection is uncommon, and occurs most frequently in the setting of immunosuppression associated with therapy for hematologic malignancies, hematopoietic cell transplantation, or solid organ transplantation [1]. The relative incidence in liver, lung and heart transplantation seems to outnumber the incidence in renal transplantation [1, 2]. This has been partly explained by the healthier preoperative state of renal transplant recipients [2].

The largest cohort of renal transplant recipients who developed invasive aspergillosis (IA) has been decribed by Baddley *et al.* and included 47 cases [3]. This study was a case series as no risk factors were described. To the best of our knowledge, the largest study investigating specific risk factors in solid organ transplantation included only 10 renal transplant recipients [4].

As IA is associated with a significant crude mortality rate [3, 5], a better understanding of the risk factors and prognostic factors are of importance, to guide preventive measures, diagnostic work-up, and therapeutic decisions.

PATIENTS AND METHODS

Study patients

This retrospective study included all patients who underwent renal transplantation at the University Hospitals Leuven, Belgium. Adult kidney transplant recipients diagnosed with IA from 1995 through 2013 were identified in the computerized patient files. Patient data were collected from a prospectively maintained database and by additional chart review. Day of diagnosis was defined as the first day of antifungal therapy. Cases of IA were classified according to Hope *et al.* [6] and as "probable" or "proven" according to the revised standard criteria from the European Organization for Research and Treatment of Cancer-Mycoses Study Group [7]. The control population in this 1:2 case control study consisted of the two patients who underwent kidney transplantation immediately before and after each identified patient and did not develop IA.

Kidney transplant protocols: immunosuppression and prophylactic measures

The immunosuppressive regimen varied according the time period, with cyclosporine and azathioprine being largely replaced by tacrolimus and mycophenolate mofetil from 1999 onwards. Only 6 patients (3 cases and 3 controls) received an mTor inhibitor (sirolimus), while all patients received corticosteroids. Induction therapy (anti-thymocyte globulin or anti-interleukin-2 receptor antibodies) was given to highly immunized recipients (panel reactive antibody above 30%, North African origin, second or subsequent transplantation) and living donation [8] since 1995. Rejection episodes were treated with methylprednisolone

in a tapering regimen. In patients undergoing transplantation after 1998, prophylaxis against CMV infection with ganciclovir was given when recipient and/or donor were CMV seropositive. *Pneumocystis jirovecii* infection prophylaxis with trimethoprim-sulfamethoxazole was given to all patients in the first 3 months following transplantation. No systemic antifungal prophylaxis was used.

Diagnostic testing

Physical examination, chest radiography and/or chest CT scan and blood cultures were performed in all febrile patients at clinical suspicion of infection. Additional samples for culture were obtained as clinically indicated. Tissue and bronchoalveolar lavage (BAL) fluid samples were evaluated for the presence of fungi by use of standard histopathologic and microbiological techniques. Since 1999 the Platelia *Aspergillus* galactomannan EIA was performed on serum samples when a diagnosis of IA was suspected. Since 2003, it was performed on BAL samples as well. A galactomannan index of ≥0.5 was used to define positivity, both in serum and BAL [9, 10].

Treatment regimens for invasive aspergillosis

Before 2003, amphotericin B products were administered as standard therapy. Since 2003, voriconazole became the drug of choice. Caspofungin was used in 4 patients and posaconazole in 1 patient, due to voriconazole intolerance/toxicity or due to co-infection with resistant fungi.

Data analysis

The primary objective of the study was to determine the risk factors in kidney transplant recipients by performing a case-control study. We included the following recipient variables: age, gender, duration of renal replacement therapy (for second and subsequent transplantations the duration after failure of the previous transplant was used), smoking habits, pre-existing chronic lung disease, diabetes mellitus prior to transplantation, respiratory function measured as part of the pre-transplant work-up, total number of HLA mismatch, use of basiliximab or anti-thymocyte globulin, donor and/or recipient CMV seropositivity and its prophylaxis, and the occurrence of leukopenia (< 3000/μL) and acute rejection after transplantation. Results from the obtained cultures and biopsies were described, in addition to the galactomannan optical density index on serum and BAL samples. The use of specific immunosuppressive drugs could not be included as variable for risk factor analysis, because of time-related changes in the patients' immunosuppressive regimen. Recipients that developed IA before month 3 after transplantation were analyzed separately as 'early IA', and the recipients with IA after month 3 as 'late IA' in concordance with previous studies [4, 11].

Continuous data were expressed as the mean ± SD and 95% CI for normally distributed data and as median and interquartile range for skewed data. Discrete variables were expressed as percentages. Continuous variables were compared using Student's unpaired *t* test or the Mann-Whitney *U* test, where appropriate. Proportions were compared using the χ^2 test or the Fisher's exact test. Odds ratios (OR) were calculated by univariate binary logistic regression analysis.

The secondary objective of this study was to determine the prognostic factors of IA after kidney transplantation. Mortality at week 12 after diagnosis was used as an end-point in concordance with other studies [3, 12]. For the mortality analyses, the same host variables were included, but instead of leukopenia after transplantation, leukopenia (< 3000/µL) at the time of presentation with the infection was included. Survival at week 12 after diagnosis was estimated with Kaplan-Meier curves and compared between groups using log-rank P values. Hazard ratios (HR) were calculated by univariate Cox proportional hazards analysis. All statistical tests were 2-tailed, and the threshold of statistical significance was P <.05.

RESULTS

Clinical presentation

Forty-one cases of IA after kidney transplantation were identified between 1995 and 2013: 24 males and 17 females, with a mean age of 61 years ± 12 at diagnosis. Eleven patients had leukopenia at presentation (27%), and 7 patients (17%) had a CMV infection prior or during the infection with *Aspergillus*. Other patient characteristics are summarized in Table 1.

The distribution of IA episodes according to the time of diagnosis after transplantation is presented in Figure 1. The median time to the development of IA was 141 days (72-522). Fifteen recipients (37%) developed early IA, and 26 recipients (63%) developed late IA. The infection was proven IA in 18 recipients

(44%) and probable IA in 23 recipients (56%). Five cases developed disseminated infection (i.e., with involvement of ≥2 non-contiguous organs). Four of these presented with symptoms of invasive pulmonary aspergillosis, one presented with an intraocular infection. All patients with disseminated IA died. In four, myocardial aspergillosis could be identified postmortem. In all cases with positive cultures (38 from 41 patients), *A. fumigatus* was identified as the pathogen. In one case, there was a co-infection with *A. niger*. From the 36 patients with localized IA, 30 had invasive pulmonary aspergillosis, four had invasive bronchial aspergillosis, one cerebral abscess and one acute invasive sinusitis extending to the mastoid bone and cerebrum. In the 34 patients with IA of the lower respiratory tract, positive cultures could be obtained in 10 out of 22 sputum samples, 14 out of 22 bronchus aspirate samples, 17 out of 27 BAL samples, 0 out of 6 pleural fluid samples and 3 out of 4 bronchus biopsy samples. Microscopy of bronchus aspirate or BAL fluid could demonstrate fungal hyphae in 2 out of 12 samples. The galactomannan index was positive in 14 out of 15 BAL fluid samples (mean index of 3.9 ± 2.5). In the total patient cohort, the galactomannan index was positive in 11 out of 33 serum samples (mean index of 1.6 ± 2.9).

Risk factors

The occurrence of leukopenia after transplantation increased the risk of IA (OR, 2.345; 95% CI, 1.084-5.071; P=0.029), whereas smoking status, gender, age, diabetes mellitus prior to transplantation, chronic lung disease, duration of renal

replacement therapy prior to transplantation, total number of HLA mismatch, presence of HLA antibodies, use of induction therapy or CMV prophylaxis, poor respiratory function tests prior to transplantation, the occurrence of CMV infection and acute rejection did not (Table 1).

Recipients with early IA had a significantly longer duration of renal replacement therapy before transplantation compared to the controls (3.57 (2.02–5.15) vs. 2.04 (1.22-3.05) years; P=0.019). Leukopenia in the first 3 months after transplantation was also associated with early IA (47% vs. 21%; P=0.049). In contrast, the duration of renal replacement therapy before transplantation did not differ between patients with late IA and the controls (1.48 (1.09-3.51) vs. 2.04 (1.22-3.05) years, P=0.808). The occurrence of leukopenia was not a significant risk factor for late IA (54% vs. 21%, P=0.119). CMV positivity of the donor increased the risk of late IA (61% vs. 29%, P=0.007). There was a trend for increased risk with seropositivity of the recipient (71% vs; 47%, P=0.051), while CMV prophylaxis (50% vs. 39%, P=0.323) and CMV infection (23% vs. 15%, P=0.367) did not increase the risk. There was also a trend for increased risk of late IA for recipients with a chronic respiratory disease(19% vs. 6%, P=0.058)(Table 2).

Survival

The mortality rate at week 12 for patients with IA was 39% (73% before 2003 and 19% since 2003). The height of the serum galactomannan index predicted 12-week mortality (HR, 1.371; 95% CI, 1.123 – 1.674) (Table 3). Using ROC curve and Youden

statistic, the optimal cutoff for the serum galactomannan level to predict the risk for death was an index of 2.0. In addition, leukopenia at the time of presentation, disseminated infection and infections acquired after 2002 were associated with 12-week mortality rate (HR, 3.198; 95% CI, 1.183 – 8.649; HR, 5.080; 95% CI, 1.740 – 14.830; and HR, 0.156; 95% CI 0.053 – 0.454). There was a trend to increased mortality according to smoking status (Table 3).

DISCUSSION

Over the past decade, risk factors for solid organ transplant-associated IA and its prognostic factors have been identified [3, 4], and this has improved early diagnosis and treatment [13-16]. The relatively lower incidence among kidney transplant recipients compared to other organ transplant recipients explains the lack of studies focusing on IA in this population. By comparing 41 cases with 82-non-infected recipients, transplanted immediately before and after each case, we could identify risk factors specific for kidney transplant recipients.

The risk of IA early after transplantation increased with prolonged renal replacement therapy before transplantation. In solid organ transplantation in general, renal failure and the need of renal replacement therapy *after* transplantation are independent risk factors for IA [4]. In liver transplantation recipients it is one of the most significant risk factors for IA, leading to a 15-to 25-fold-greater risk [19, 20]. Our results demonstrate for the first time an increased

susceptibility with longer *duration* of renal replacement therapy *before* transplantation.

Similar to previous studies in solid-organ transplantation, leukopenia increased the risk for IA [4]. We choose a less strict definition of leukopenia compared to studies in haematology to determine risk factors specific for the transplant setting. This is in agreement to a similar case-control study on IA in solid-organ transplantation [4]. The retrospective design of this study precluded us to obtain information about duration of leukopenia.

There was a trend to an increased risk for late IA in recipients with a chronic respiratory disease. Others have previously suggested an association between respiratory dysfunction and risk of IA, as patients with chronic obstructive pulmonary disorder (COPD) are at risk of invasive pulmonary aspergillosis and their increased susceptibility does not seem to be explained by exposure to steroids alone [5, 21-23].

In line with previous reports [24], we did not find an association between acute rejection and the occurrence of IA. We did find an association between donor CMV positivity and the risk for late IA, similar to what has been observed in liver transplant recipients [25]. CMV infection is known to induce an immunosuppressed state [26] and its therapy can induce leukopenia. In liver transplant recipients CMV disease is associated with invasive fungal disease [25]. In our cohort, 17% of cases had a CMV infection immediately prior or during the infection with *Aspergillus*.

However, the occurrence of CMV infections after transplantation was not significantly higher compared to the control group.

The overall 12-week mortality of 39% is lower than the mortality previously described among HSCT and transplant patients [27][5], but comparable to a more recent study in solid organ transplantation [3]. Mortality decreased after 2002, when voriconazole was introduced in our therapy regimens. The prognostic effect of this time point was more pronounced than the effect of treatment regimens including voriconazole. However, this study was not designed to investigate the prognostic role of a specific antifungal therapy. In addition, many patients received subsequently different regimens, for example due to renal function deterioration. Patients with leukopenia at presentation had an increased risk of death. A higher case fatality rate in patients with neutropenia has already been observed [5], but has not been reported for solid organ transplant recipients in specific [4]. In addition, the height of the serum galactomannan test correlated with mortality, whereas the BAL galactomannan did not. This is in agreement with what has been reported in HSCT patients [28] and patients with hematologic cancer [29]. We are the first to describe this association in solid organ transplant recipients. In contrast, the serum galactomannan test lacks sensitivity for IA diagnosis in solid organ transplant recipients, whereas the sensitivity of the galactomannan test in BAL fluid is high in this population [16, 30, 31]. The higher serum galactomannan level is thought to represent a higher Aspergillus burden and/or increased micro- or macro-

dissemination [28]. In our cohort, disseminated IA increased the risk of death, similar to what has been reported elsewhere [3, 12, 27].

We could not observe an association between the respiratory function measured pre-transplant and mortality after IA. In a study of HSCT patients, a compromised respiratory function pre-transplant was the only host factor independently associated with an increased risk for death, but the criteria used to define severe pulmonary function abnormality were not reported in this study [27].

The strength of our study is the relatively large case cohort among kidney transplant recipients compared to other studies, the focus on kidney transplantation alone and the inclusion of only proven and probable cases. However, the number of cases was too low to perform accurate multivariate testing. In addition, the retrospective design in this large time frame precluded the collection of all variables in all patients.

In conclusion, the duration of renal replacement therapy and the occurrence of leukopenia increased the risk for early IA after kidney transplantation. Mortality rate was highest among recipients with leukopenia, disseminated infection and higher levels of serum galactomannan test.

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Potential conflicts of interest:

JM has served as consultant to Schering-Plough, Gilead Sciences, Merck, Sharp & Dohme, Pfizer Inc., and Astellas; has received research funding from Merck, Sharp & Dohme, Pfizer Inc, Gilead Sciences and Astellas, and has been on the speaker's bureau for Schering-Plough, Gilead Sciences, Merck, Sharp & Dohme, Pfizer Inc., and Astellas.

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Table 1: Patient characteristics. Continuous variables were compared using Student's unpaired *t* test or the Mann-Whitney *U* test, where appropriate. Proportions were compared using the χ^2 test or the Fisher's exact test, where appropriate.

Variable	cases (n=41)	controls (n=82)	P-value
Gender (male/female)	22/19	50/32	0.734
Age[1] (years)	58 ± 12	55 ± 12	0.155
Smoking[1] (yes/no)	5/33 (15%)	5/79 (6%)	0.156
Diabetes mellitus[1] (yes/no)	10/41 (24%)	15/82 (18%)	0.428
Chronic respiratory disorder[1] (yes/no)	7/41 (17%)	5/82 (6%)	0.102
Duration RRT[1] (years)	2.85 (IQR 2.53)	2.04 (IQR 1.83)	0.268
Total number of HLA mismatch	3 ± 1	3 ± 2	0.375
HLA antibodies	3/39 (8%)	6/80 (8%)	1.000
CMV positive recipient	23/36 (64%)	34/72 (47%)	0.102
CMV positive donors	16/38 (42%)	22/74 (30%)	0.190
CMV D+/R-	5/35 (14%)	12/70 (17%)	0.708
CMV prophylaxis	22 (54%)	32 (39%)	0.123
Induction therapy	13/41 (32%)	20/82 (24%)	0.388
FEV1 (%)	86 ± 20	91 ± 20	0.234
FEFV1/FVC	77 ± 7	80 ± 10	0.216
TLC (%)	93 ± 15	98 ± 21	0.283
DLCO (%)	69 ± 20	72 ± 20	0.520
CMV infection[2]	8/41 (22%)	12/82 (15%)	0.309
Acute rejection[2]	14/40 (35%)	23/82 (28%)	0.290
Leukopenia[2]	22/41 (54%)	30/82 (37%)	0.029

NOTE [1]At transplantation time point, [2]At any time point post-transplant

RRT, renal replacement therapy; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusion capacity

Table 2: Risk factors for early and late invasive aspergillosis by univariate logistic regression analysis.

	Early IA		Late IA	
Variable	P-value	OR (95% CI)	P-value	OR (95% CI)
Gender	0.305	1.786 (0.590 - 5.403)	0.766	1.146 (0.468 - 2.806)
Age[1]	0.281	1.028 (0.977 - 1.082)	0.255	1.024 (0.983 - 1.066)
Smoking[1]	0.190	3.289 (0.555 - 19.499)	0.273	2.337 (0.512 - 10.658)
Diabetes mellitus[1]	0.456	1.624 (0.454 - 5.807)	0.592	1.340 (0.459 - 3.908)
Chronic respiratory disorder[1]	0.332	2.369 (0.415 - 13.525)	0.056	3.667 (0.970 - 13.866)
Duration RRT (years)	**0.042**	**1.192 (1.006 - 1.413)**	0.564	0.935 (0.744 - 1.175)
Total HLA mismatch	0.271	1.239 (0.846 - 1.815)	0.712	1.059 (0.781 - 1.437)
HLA antibodies	0.910	0.881 (0.098 - 7.893)	0.893	1.121 (0.211 - 5.954)
CMV positive recipient	0.667	1.277 (0.419 - 3.895)	0.056	2.794 (0.974 - 8.015)
CMV positive donor	0.207	0.364 (0.076 - 1.748)	**0.009**	**3.677 (1.388 - 9.743)**
CMV D + R -	0.326	0.345 (0.041 - 2.882)	0.768	1.208 (0.343 - 4.259)
Use of CMV prophylaxis	0.305	1.786 (0.590 - 5.403)	0.324	1.562 (0.643 - 3.796)
Induction therapy	0.216	2.067 (0.655 - 6.523)	0.795	1.142 (0.419 - 3.112)
FEV1 < 90%	0.068	3.720 (0.909 - 15.220)	0.988	0.992 (0.341 - 2.888)
FEV1/FVC < 70	0.883	1.136 (0.209 - 6.159)	0.504	1.573 (0.416 - 5.940)
TLC < 90%	0.928	1.071 (0.242 - 4.743)	0.339	1.750 (0.555 - 5.514)
DLCO < 60%	0.990	1.010 (0.232 - 4.399)	0.533	1.496 (0.422 - 5.300)
FEV1/FVC < 70 or TLC < 90% or DLCO < 60%	0.526	1.548 (0.401 - 5.975)	0.227	2.212 (0.610 - 8.019)
CMV infection[2]	0.999	/	0.318	1.750 (0.583 - 5.251)
Acute rejection[2]	0.084	2.712 (0.874 - 8.420)	0.996	0.998 (0.368 - 2.704)
Leukopenia[2]	**0.039**	**3.346 (1.063 - 10.527)**	0.122	2.022 (0.828 - 4.936)

NOTE [1]At transplantation time point, [2]In the first 3 months post-transplant for early IA risk factor analysis and at any time point post-transplant for late IA risk factor analysis.

RRT, renal replacement therapy; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusion capacity

Table 3: Risk factors for week 12 mortality of invasive aspergillosis by univariate Cox proportional hazards analysis.

Variable	P-value	HR (95% CI)
Gender	0.460	1.448 (0.543 - 3.863)
Age at diagnosis of IA	0.111	0.970 (0.934 - 1.007)
Smoking[1]	0.069	3.457 (0.909 - 13.143)
Diabetes mellitus[1]	0.075	0.159 (0.021 - 1.202)
Chronic respiratory disorder[1]	0.201	0.267 (0.035 - 2.023)
Duration RRT (years)	0.453	1.063 (0.906 - 1.248)
Total HLA mismatch	0.749	1.061 (0.737 - 1.528)
HLA antibodies	0.578	1.523 (0.346 - 6.710)
CMV positive recipient	0.180	0.511 (0.192 - 1.365)
CMV positive donor	0.154	2.052 (0.763 - 5.516)
Use of CMV prophylaxis	0.664	1.251 (0.455 - 3.443)
Induction therapy	0.347	0.581 (0.187 - 1.802)
FEV1 < 90%	0.320	0.547 (0.167 - 1.796)
FEV1/FVC < 70	0.228	0.030 (0.000-8.969)
TLC < 90%	0.987	1.010 (0.285 - 3.584)
DLCO < 60%	0.938	1.057 (0.264 - 4.233)
FEV1/FVC < 70 or TLC < 90% or DLCO < 60%	0.789	0.827 (0.207 - 3.313)
CMV infection prior or during IA	0.884	0.896 (0.203 - 3.947)
IA following acute rejection	0.257	1.774 (0.658 - 4.778)
Leukopenia at clinical presentation of IA	**0.022**	**3.198 (1.183 - 8.649)**
Disseminated vs. localized IA	**0.003**	**5.080 (1.740 - 14.830)**
Proven vs. probable IA	0.104	2.319 (0.841 - 6.393)
Early vs. late IA	0.971	0.981 (0.356 - 2.700)
GM index in serum	**0.002**	**1.371 (1.123 - 1.674)**
GM index in BAL	0.243	1.742 (0.687 - 4.421)
IA after 2002	**0.001**	**0.156 (0.053 - 0.454)**
Therapy containing voriconazol	0.079	0.340 (0.102 - 1.133)

NOTE [1]At transplantation time point, [2]At any time point post-transplant RRT, renal replacement therapy; FEV1, forced expiratory volume in 1 second; FVC, forced vital capacity; TLC, total lung capacity; DLCO, diffusion capacity; IA, invasive aspergillosis; GM, galactomannan; BAL, bronchoalveolar lavage.

Figure legends:

Figure 1: Distribution of the time frame between kidney transplantation and diagnosis of invasive aspergillosis.

Figure 2: Kaplan Meier curves for 12 week survival according to A. galactomannan level (GM) in serum above or below 2, B. leukopenia at presentation, C. disseminated nature of the infection.

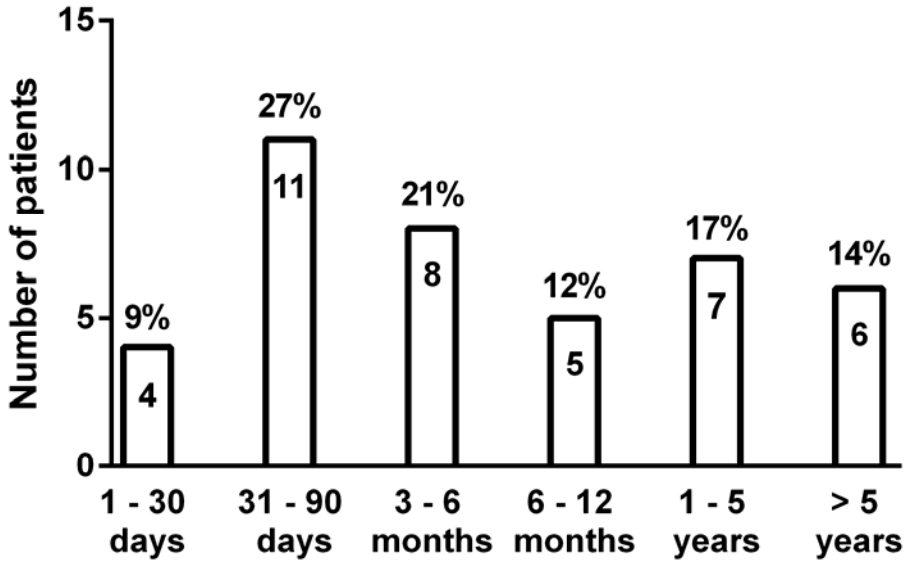

Figure 1.

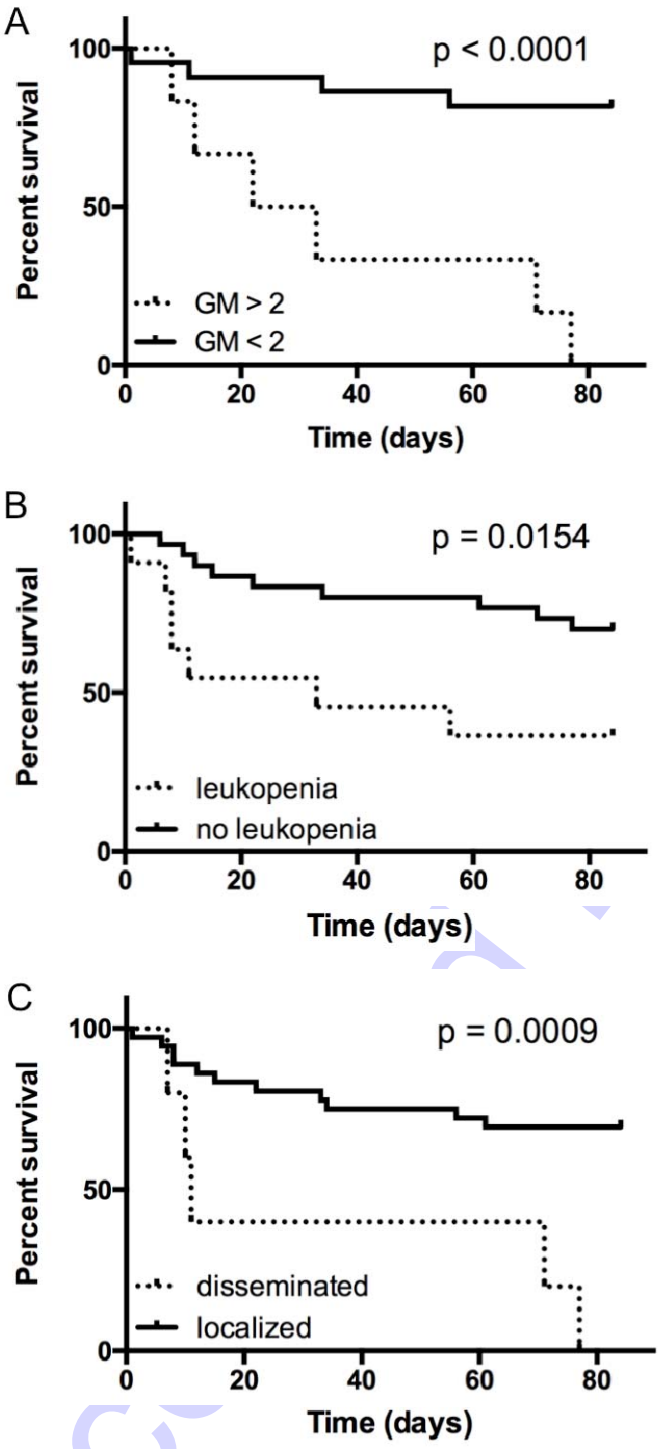

Figure 2.