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Risk factors for post-engraftment invasive aspergillosis in allogeneic stem cell transplantation

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Summary:

The majority of invasive aspergillosis (IA) in allogeneic stem cell transplantation (SCT) occurs during the post-engraftment period. We used Cox proportional hazards regression to evaluate post-engraftment IA risk in a cohort of 217 allogeneic SCT recipients from 1991 to 1998. The aim was to quantify the effects of dose-intensity and duration of corticosteroids and other risk factors. Median duration of follow-up was 330 days. There were 19 cases of IA (overall 8.8%) with 14 post-engraftment infections. In the final model, the risk of IA was greatest within 2 weeks of high-dose corticosteroids (HR 8.5, $P = 0.003$), with risk extending to 4 weeks with doses of 0.25–1 mg/kg/day (HR 3.1, $P = 0.08$). Ganciclovir was associated with greatest risk (HR 13.6). Grade 3 or 4 acute GVHD (HR 5.7) and secondary neutropenia (HR = 1.3) were also additive risks. In the univariate analysis, corticosteroid doses of 0.25–1.0 mg/kg/day for any duration between 2 and 10 weeks demonstrated prolonged risk for IA. Moderate doses of corticosteroids can confer an increased risk for IA for extended periods which is almost as marked as that conferred by higher doses. Knowledge of these risks may facilitate the development of targeted surveillance and prophylaxis strategies for prevention of IA.
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Throughout the 1990s the incidence of late invasive aspergillosis (IA) after allogeneic stem cell transplantation (SCT) increased such that now most IA is observed in the post-engraftment period.[1–3] The introduction of reduced intensity conditioning regimens, peripheral blood stem cells and newer antifungal agents have not appeared to decrease the risk of late IA.[4–6] Current antifungal prophylactic strategies have had little effect on the incidence of IA[7,8] and once the diagnosis is made, survival is universally poor. Targeted surveillance and/or use of newer agents for antifungal prophylaxis in high-risk patients may improve outcome if such patients can be reliably predicted.

The major risk factors for late IA are graft versus host disease (GVHD), the use of corticosteroids and cytomegalovirus (CMV) disease.[2–4,9] Although several authors have demonstrated significant risk associated with higher dose corticosteroids given for defined durations,[2–4,9,10] the duration of risk after exposure and the relationship of risk to dose/intensity have not been clearly defined. In this study, we examine the effects of both dose intensity and duration of corticosteroid use, other immunosuppressants, antimicrobials and other known risk factors on the risk of post-engraftment IA in a cohort of allogeneic SCT recipients.

Methods

Patients

The medical records of all adult patients who underwent allogeneic SCT at the Royal Melbourne Hospital (Victoria, Australia) from January 1991 to June 1998 were reviewed. For each patient data were collected from the date of transplant to the last day of documented follow-up or death. Data were collected up to June 2000. Information about prior fungal infections was also recorded, although only IA infections occurring after transplantation, after engraftment were included in the analysis.

Transplantation protocols

Graft-versus-host-disease prophylaxis and treatment. Standard prophylaxis for GVHD included the use of cyclosporine and short-course methotrexate. Corticosteroids were not used prophylactically, being utilized for grade 2–4 acute GVHD or extensive chronic GVHD. While the corticosteroid doses were individualized according to the severity of GVHD and response to treatment, generally the starting dose was methylprednisolone 2 mg/kg i.v. for gastrointestinal GVHD and prednisolone 1 mg/kg orally for cutaneous GVHD. Subsequent doses were adjusted according to

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clinical response. Antithymocyte globulin (ATG) was added for GVHD refractory to at least 10 days of high-dose corticosteroids (>1 mg/kg/day prednisolone equivalent). T-cell depletion was not used.

Antifungal prophylaxis and treatment. All patients were managed in HEPA-filtered rooms from the start of conditioning to engraftment. Fluconazole was used as antifungal prophylaxis at a dose of 400 mg/day from the onset of neutropenia to neutrophil recovery, then 200 mg/day until day 100. Beyond day 100, patients receiving a corticosteroid dose of greater than 20 mg of prednisolone daily continued to receive fluconazole. From November 1997, sibling allograft recipients receiving more than 3 weeks of $\geqslant$50 mg prednisolone per day (2 weeks in recipients of mismatched related or unrelated grafts) had fluconazole prophylaxis replaced by itraconazole cyclodextrin solution 2.5 mg/kg twice daily until the prednisolone dose was less than 10 mg/day. Itraconazole levels were monitored.

Antibiotic prophylaxis. All patients received trimethoprim–sulphamethoxazole or pentamidine prophylaxis for PCP until 6 months post transplant or 3 months after the end of immunosuppressive therapy if this was given for periods exceeding 6 months. Phenoxymethylpenicillin 500 mg daily was given if there was chronic GVHD.

Antiviral prophylaxis and treatment. All patients seropositive for herpes simplex virus or varicella zoster virus received acyclovir 400 mg orally twice daily or dose 250 mg i.v. three times daily from onset of neutropenia until hospital discharge. Patients who were seropositive for CMV or receiving marrow from a seropositive donor received intravenous ganciclovir prophylaxis at a dose of 5 mg/kg three times weekly from engraftment to day 100 post transplant. Beyond day 100, patients with ongoing GVHD requiring $\geqslant$20 mg/day or more of prednisolone had ganciclovir prophylaxis continued until the corticosteroid dose prescribed was below 20 mg. Acyclovir 200 mg three times daily orally was given in HSV or VZV seropositive patients not receiving ganciclovir until 3 months after the end of immunosuppressive therapy. CMV disease was treated with at least 2 weeks standard of ganciclovir induction therapy until clearance of virus followed by maintenance therapy.

Data collection and definitions. Baseline information recorded for each patient included age, gender, underlying disease, disease status (first remission, relapse/refractory, untreated), transplant type (related, unrelated, mismatched unrelated), conditioning regimen (myeloablative *vs* reduced intensity), and donor/patient CMV status. Two dedicated study nurses recorded the start date, stop date, and daily dose of the following drugs: corticosteroids, cyclosporin, antifungal agents, acyclovir, ganciclovir, ATG, tacrolimus, other immunosuppressants and filgrastim. Corticosteroid doses were converted to prednisolone equivalents, and oral ganciclovir was converted to i.v. doses based on oral bioavailability data.[11] Every attempt was made to retrieve complete data about outpatient dosing of drugs up to 2 years post transplant or last day of follow-up/death.

Acute and chronic GVHD were classified according to published criteria.[12,13,14] Sites of acute and chronic GVHD were recorded. Proven CMV disease and site of infection were also collected. CMV disease was defined as per published criteria.[15]

Time to engraftment was defined as time from transplant until the neutrophil count exceeded 0.5×10^9/l. Secondary neutropenia was defined as 2 or more consecutive days of neutropenia less than or equal to 0.5×10^9/l. For patients who failed to engraft, secondary neutropenia was counted after day 30 post transplant.

Microbiology, pathological, clinical and radiological features were reviewed to assess the likelihood of IA. Fungal infections were defined as proven or probable according to criteria proposed by Ascioglu *et al*.[16] Standard microbiological methods for direct microscopy, culture, and identification of fungi were used. The day of suspected onset was defined as the day on which symptoms first developed or treatment was started empirically whichever was earlier. The date of confirmation was defined as the day on which a positive culture was obtained; in some cases, this was at post-mortem. Date and cause of death were recorded.

Statistical analysis

The analysis used Cox proportional hazards regression with the day of suspected onset of probable or proven IA as failure. Time was counted from the day of transplant.

In the absence of a convincing *a priori* hypothesis for the duration of corticosteroid effects, we undertook an exploratory analysis. The covariates for corticosteroid use were defined by dose and time intervals received before suspected onset of IFI. Daily doses of corticosteroids as prednisolone equivalents (PNE) were stratified into three dose ranges; <0.25 mg/kg/day, 0.25–1 mg/kg/day and >1 mg/kg/day (among all days, including those where no corticosteroids were given). These dose ranges corresponded to the 0–50, 50–95, and >95th percentiles respectively. The duration of exposure was defined by 2-weekly intervals (1–13, 14–27, 28–41, 42–55, and 56–69 days) prior to failure. In a Cox regression analysis, each patient may enter the analysis several times: while acting as a control for other patients who fail while the index case is at risk, and at his or her own failure, should it occur.[17] It is important that the covariates reflect the circumstances of the patients at each of these times. Thus 'steroid dose 0–13 days prior' refers to steroids received in the 2 weeks prior to the time defining each risk set.

The hazard ratio was estimated separately for each time interval out to 10 weeks prior to the day of analysis. As doses in one period are predictive of doses received in adjacent periods, leading to confounding, a separate multivariate analysis was performed for each covariate to determine the independent effect due to doses received in each period.

Hazard ratios were calculated for patient age, sex, underlying malignancy, conditioning regimen, transplant type, CMV serostatus, CMV infection, and graft-versus-

host-disease. For filgrastim, acyclovir, ganciclovir, fluconazole, and cyclosporin, doses received 0–13 and 14–27 days prior to suspected infection were analysed. The use of itraconazole and amphotericin B were excluded from the analysis, as the uses of these agents were markers for high-risk patients in our unit. Cyclosporin was stratified according to doses per weight exceeding the 90th percentile of all doses. Filgrastim, fluconazole, acyclovir and ganciclovir were analysed as categorical variables (yes/no). The presence of secondary neutropenia was analysed in 0–13 and 14–27 day intervals. As for the corticosteroid covariates, a bivariate analysis was performed for adjacent time intervals.

The grades of acute GHVD were collapsed into 0–2 and 3–4 to make data more interpretable. As there were no cases of IA in the limited chronic GVHD group, the regression was stratified by limited chronic GVHD.

The factors included in the multivariate model for backward selection were all those reaching a liberal standard of significance ($P<0.25$) in the univariate or bivariate analyses. A global test for the proportional hazards was tested and accepted ($P=0.94$) for the final multivariate analysis. Statistical calculations were performed with the use of STATA software (version 7).[18]

Results

Of 229 patients identified as undergoing allogeneic SCT between 1991 and 1998, the records of 217 were available. The proportion of allogeneic SCT patients with proven or probable IA ranged from 8–14% per year during the study period. The overall proportion was 8.8%. The median time to suspected infection from the date of SCT was 72 days (6–545). Once IA was suspected, the median time to confirmation was 7 days (0–51). The median time to death once IA was suspected was 19 days (1–411). For 13 patients, IA was the main cause of death (68% attributable mortality), two survived and four died of other causes. All cases had pulmonary involvement and three were disseminated. *Aspergillus fumigatus* was isolated in 14 cases, and Aspergillus nonspeciated was isolated in five cases. The median duration of follow-up was 330 days (2–2194 days).

Risk factors for post-engraftment invasive Aspergillus infection

Of 19 cases of proven ($n=15$) or probable IA ($n=4$) diagnosed post transplant, 14 cases occurred in the post-engraftment phase and were included in the analysis. Six patients with a diagnosis of IA before transplant were excluded from the analysis. The total number of patients remaining in the post-engraftment cohort was 206.

Unadjusted hazard ratios for IA risk were calculated for patient- and disease-related factors (see Table 1). The presence of grade 3 or 4 acute GVHD was most strongly associated with the risk of IA. Age, sex, underlying disease, disease status, conditioning regimen, and year and season of transplant were not associated with IA risk.

Data on secondary neutropenia was available for 192 patients (93%). At least one episode occurred in 90 patients. Secondary neutropenia was a risk factor if present between 0 and 13 days but not 14 and 27 days prior to IA.

A separate analysis was performed to identify the most significant time intervals and dose ranges for corticosteroids. The median dose of prednisolone equivalents for each interval was 0.2 mg/kg/day. Table 2 lists unadjusted hazard ratios for patients receiving corticosteroids in periods before onset of suspected IA. A dose response is observed between time from IA and dose level (see Figure 1). Prednisolone doses of at least 1 mg/kg/day were strongly associated with risk of IA if received within 4 weeks of suspected IA. Doses of 0.25–1 mg/kg/day for up to and including 10 weeks were associated with an increased risk. A backwards-stepwise regression was performed to identify those variables independently associated with IA. Doses of >1 mg/kg/day 0–2 weeks and 0.25–1 mg/kg/day 2–4 weeks and 6–8 weeks prior to IA were retained and included in the final model.

Table 3 lists unadjusted hazard ratios for the remaining covariates. There were no cases of IA in patients who received ATG ($n=12$). There were also no cases in patients receiving greater than 200 mg cyclosporin per day. Acyclovir or fluconazole received in the month before IA was not a risk factor. Filgrastim was a risk factor if received 1–2 weeks before IA.

In this cohort of patients, 29% of patients with IA had CMV disease compared to 7% in the uninfected group. CMV disease significantly increased the risk for IA (HR 3.9, 95% CI 1.2–12.6) on univariate analysis but dropped out in the final model. The use of any ganciclovir was the strongest risk factor for IA on both univariate and multivariate analysis (although confidence intervals were wide).

In the final Cox proportional hazards model shown in Table 4, five variables were independent predictors for post-engraftment IA. The use of ganciclovir remained the strongest risk factor independent of proven CMV disease followed by the use of high-dose prednisolone (>1 mg/kg/day) within 0–13 days and grade 3 or 4 acute GVHD. Low-dose prednisolone (0.25–1 mg/kg/day) within 14–27 days approached significance (HR 3.1, 95% CI 0.87–11.07, $P=0.80$). Secondary neutropenia was the weakest risk factor (HR 1.3), but statistically very significant.

Discussion

In this cohort of allogeneic SCT recipients predominantly receiving myeloablative conditioning regimens, the incidence and timing of IA was similar to that described by other groups.[1–3] This study provides new data on the threshold doses of corticosteroids associated with risk for IA as well as the extended risk periods associated with and following cessation of corticosteroid use in the treatment of GVHD. It also confirms an earlier finding of the potential risks associated with ganciclovir use.

One of the strengths of this study is the detailed and extended data collection (at least 1 year) for corticosteroid

Table 1 Characteristics of patients undergoing allogeneic SCT at RMH from 1991 to 1998 with associated risks for post-engraftment invasive aspergillosis

Characteristic	*No IA Total = 192 N (%)*	*IA Total = 14 N (%)*	*Hazard ratio*	*95% CI*	*P-value*
Median age at transplant (years)	39 (16–69)	41 (16–55)			
Age >40 years	86 (45)	8 (57)	1.40	0.48–4.05	NS
Sex Male	112 (58)	12 (86)	3.84	0.86–17.17	0.08
Diagnosis					
AML	54 (28)	3 (21)	0.64	0.18–2.31	NS
CML	52 (27)	3 (21)	1.18	0.24–5.86	NS
ALL	32 (17)	3 (21)	1.97	0.39–9.80	NS
Multiple myeloma	9 (5)	0	—	—	—
Myelodysplasia	12 (6)	1 (7)	1.07	0.11–10.37	NS
Other	32 (17)	4 (30)	2.21	0.49–9.92	NS
Disease status at BMT					
Remission	27 (14)	1 (7)	—	—	—
Refractory/relapse	155 (81)	12 (86)	2.1	0.3–15.6	NS
Untreated	10 (5)	1 (7)	1.22	0.16–9.46	NS
Donor type					
Related	160 (81)	11 (64)			
Unrelated	32 (17)	3 (21)	0.78	0.21–2.88	NS
Conditioning regimen[a]					
Myeloablative	175 (90)	11 (79)			
Non-myeloablative	15 (8)	3 (21)	0.48	0.1–1.76	NS
Time to engraftment (days)					
Median (range)	16 (2–30)	19 (4–29)	0.57	0.18–1.85	NS
CMV status[b]					
D+ R+	67 (35)	6 (42)	1.74	0.59–5.04	NS
D+ R−	26 (14)	0	—	—	—
D− R+	50 (26)	3 (21)	0.60	0.18–2.08	NS
D− R−	41 (21)	5 (37)	2.00	0.66–6.05	NS
CMV disease	13 (7)	4 (29)	3.90	1.21–12.56	0.02
Acute GVHD[c]					
0–2	172 (90)	7 (50)	1.0	—	—
3–4	20 (10)	7 (50)	8.75	3.01–25.40	<0.0001
Chronic GVHD[d]					
Absent	49 (26)	6 (43)	1.0	—	—
Limited	62 (32)	0	—	—	—
Extensive	52 (27)	8 (57)	1.18	0.41–3.42	NS

[a]Regimen missing for two patients. Myeloablative regimens included predominantly busulphan/cyclophosphamide(cy), cy-total-body irradiation (TBI) and VP16-TBI. Nonmyeloablative regimens included predominantly fludarabine–melphalan, fludarabine–cyclophosphamide regimens. T-cell depletion was not used. ATG was only used in aplastic anaemia ($n = 12$).
[b]CMV serostatus not available for nine patient/donor pairs.
[c]GVHD status unknown for two cases.
[d]Includes patients alive and at risk >d100.

and other analysed drugs in the post transplant period. The effect of individualized clinical practice, in which a patient may have started or stopped a course of corticosteroids in the days or weeks before IA developed is captured. This is of relevance as in our study the median onset of IA was day 72 with 7/19 cases occurring beyond day 100 post transplant. This was also the case in other studies with the median day of onset reported as day 92 and 102 post transplant.[3,4] Both the daily dose and duration of corticosteroid use determine the incidence of infectious complications.[19] To our knowledge, this is the only study that has quantified the extended risk of post-engraftment IA in relation to dose intensity and duration of corticosteroids.

In the final model, the risk of IA was greatest within 2 weeks of high-dose steroids, with risk extending to 4 weeks with doses of 0.25–1 mg/kg/day. Higher doses of >1.0 mg/kg/day demonstrated a dose–response effect extending to 6 weeks. In the univariate analysis, corticosteroid doses of 0.25–1.0 mg/kg/day for any duration between 2 and 10 weeks demonstrated a prolonged risk for IA. Hence, it appears that relatively moderate doses of corticosteroids

may be associated with a prolonged risk of IA. This dose is lower than the doses independently associated with IA risk in previous studies.[2–4] This finding, unique to our study, may be explained by the collection of daily doses, prolonged duration of follow-up and type of analysis used.

Differing statistical analyses have been used to quantify the relationship between corticosteroid use and IA in SCT. Two studies have used logistic regression to demonstrate the risk associated with a period of steroid use exceeding a dose threshold. Significant corticosteroid use was defined as >30 days at 0.5 mg/kg/day by Baddley[4] and as >21 days at >1 mg/kg by Grow *et al*.[3] Logistic regression quantified the risk of IA associated with these variables; however, these studies did not provide information about the duration of risk associated with corticosteroids.

In a recent study,[2] the highest daily dose of corticosteroids from day 41 to 100 post transplant was analysed as a time-dependent variable. Doses of 2.0–2.9 mg/kg/day (HR = 8.0) and >3.0 mg/kg/day (HR 15.4) increased the risk of late IA; however, inferences cannot be made about cases that occurred after day 100 due to the censoring of steroid data beyond that day.

In our study, the use of ganciclovir was associated with significant IA risk, independent of secondary neutropenia and proven CMV infection. There is a complex interrelationship between GVH, CMV reactivation, ganciclovir exposure, and development of IA. The combination of ganciclovir and CMV disease has been postulated to cause both immuno- and myelosuppression.[20,21] Einsele *et al*[22] demonstrated that extended antiviral therapy with ganciclovir (⩾4 weeks) was associated with substantial risks for bacterial and fungal infections and late-onset CMV disease (due to a delayed recovery of CMV-specific T-cell responses). The risk of IA increased by a factor of 1.4 for each week of treatment. Late CMV infection was only seen

Table 2 Hazard ratios for IA according to prednisolone equivalents stratified by time intervals before IA and dose

PNL equivalent (mg/kg/day)	*Interval before IA (days)*	*Hazard ratio*	*95% CI*	*P-value*
Any	0–13	2.30	1.43–3.70	0.001
<0.25		1.00	—	—
0.25–1.0		3.63	0.86–15.28	0.08
>1.0		13.52	2.95–61.94	0.001
Any	14–27	1.85	1.09–3.12	0.02
<0.25		1.00	—	—
0.25–1.0		5.85	1.62–21.16	0.007
>1.0		6.33	0.99–40.19	0.05
Any	28–41	1.43	0.84–2.44	0.19
<0.25		1.00	—	—
0.25–1.0		2.72	0.70–10.56	0.15
>1.0		2.85	0.49–16.57	0.24
Any	42–55	1.03	0.53–1.98	0.10
<0.25		1.00	—	—
0.25–1.0		3.55	1.10–11.36	0.03
>1.0[a]		—		—
Any	56–69	0.96	0.47–1.96	0.94
<0.25		1.00	—	—
0.25–1.0		3.20	0.93–11.02	0.06
>1.0[a]		—		—

[a]There were no cases of IA in patients in these categories.

Table 3 Bivariate Cox regression analysis of cyclosporin, antivirals, filgrastim, cyclosporin, and secondary neutropenia

Variable	*Interval before IA (days)*	*Hazard ratio*	*95% CI*	*P-value*
Cyclosporin >200 mg/day	0–13	—	—	NS
	14–27	—	—	NS
Any acyclovir	0–13	0.88	0.25–3.10	NS
	14–27	1.96	0.61–6.34	NS
Any ganciclovir	0–13	11.18	2.45–50.85	0.002
	14–27	17.21	3.53–84.01	<0.0001
Any fluconazole	0–13	1.08	0.29–4.10	0.91
	14–27	1.00	0.99–1.00	0.16
Any filgrastim	0–6	3.31	0.67–16.48	NS
	7–13	7.39	1.83–29.80	0.005
Secondary neutropenia[a]	0–13	1.26	1.06–1.50	0.009
	14–27	0.88	0.56–1.39	NS

[a]Secondary neutropenia data was unavailable for 14 patients (one of these was an IA case).

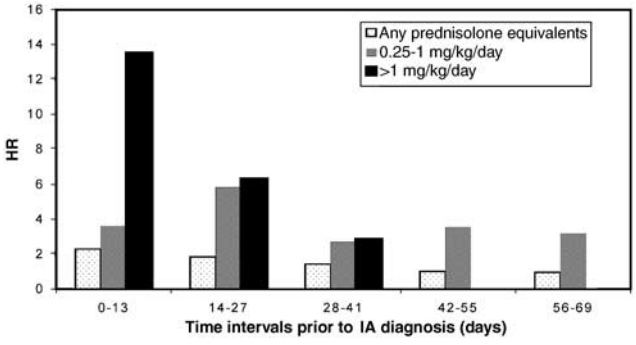

Figure 1 Risk of invasive aspergillus (as hazard ratios (HR) after receiving prednisolone in stratified doses and time intervals.

Table 4 Final model showing additive risks for invasive aspergillosis

Variable (time interval prior to IA)	*Hazard ratio*	*95% confidence interval*	*P-value*
Any ganciclovir (14–27 days)	13.6	2.37–77.81	0.003
Prednisolone equivalent >1 mg/kg/day (0–13 days)	8.5	20.2–35.55	0.003
Grade 3 or 4 acute GVHD	5.7	1.87–17.55	0.002
Prednisolone equivalent >0.25–1 mg/kg/day (14–27 days)	3.1	0.87–11.07	0.08
Secondary neutropenia (0–13 days)	1.3	1.07–1.52	0.006

in the subset of patients with cGVHD. A recent study identified CMV reactivation within 3 months, poor CMV-specific immunity and low CD4 cell counts as the main risk factors for late CMV infection.[20] In these patients, there is a role for continued CMV screening (after day 100 post HSCT) and pre-emptive therapy to limit total ganciclovir exposure. Reducing ganciclovir use may reduce the risk of IFI. It would also seem prudent to target antifungal prophylaxis or surveillance to these patients.

There are a number of factors that may limit the generalizability of these results. This single centre study was retrospective, nonrandomized and conducted predominantly in myeloablative SCT recipients. The variability of the confidence intervals relates to small sample size, one of the inherent problems in studying rare diseases such as IA. At the time of the analysis, there were no data regarding the utility of itraconazole prophylaxis in allograft recipients and many centres did not use it or used it according to individualized protocols. Itraconazole was excluded from the analysis although it was used as prophylaxis for selected high-risk patients as described in the Methods section. Of 20 patients in this cohort who received itraconazole, four went on to develop late IA (twice the expected rate). As used in our unit, itraconazole was clearly a marker for high risk for IA and any findings would not be relevant to other centres. In contrast, fluconazole prophylaxis was analysed as its utility has been demonstrated[23] and at the time of this analysis its use was a widely accepted practice (although the use of fluconazole prophylaxis beyond day 100 in patients receiving >25 mg of prednisolone a day may not reflect the practises of other centres).

The final Cox proportional hazards model shows additive risks for corticosteroid, ganciclovir use grade 3 or 4 acute GVHD and secondary neutropenia. It provides threshold corticosteroid doses for an extended risk of IA, which have not been previously reported. It also provides evidence that even modest doses of corticosteroid (0.25–1 mg/kg/day) over a 4–6 week period confers an increased risk for IA which is almost as marked as that conferred by higher doses. This model allows a more individualized ascertainment of risk for IA and assessment of duration of ongoing risk. Knowledge of these risks may facilitate the development of targeted surveillance and prophylaxis strategies for the prevention of IA. A limitation to the utility of surveillance for late IA with PCR or antigen detection kits has been the logistics of monitoring all at-risk patients over the prolonged period of risk. Such models will be useful in defining which patients should be followed with these tests and for how long. Further work is required to validate this model and it will be applied to another cohort of allogeneic SCT patients to evaluate its predictive status.

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