GENOTYPING OF HUMAN CYTOMEGALOVIRUS (HCMV) GLYCOPROTEIN B (gB) IN HEMATOPOIETIC STEM CELL TRANSPLANTATION RECIPIENTS WITH ACTIVE HCMV INFECTION: IMPACT OF gB GENOTYPES ON THE PATIENT’S OUTCOME


Based on sequence variation in the UL55 gene that encodes glycoprotein B (gB), human cytomegalovirus (HCMV) can be classified into four gB genotypes. There is little information about the CMV gB genotype and clinical outcome in patients who underwent an allogeneic hematopoietic stem cell transplant (HSCT) in Brazil.

Objectives: The goal of this study was to determine the distribution of gB genotypes in allogeneic HSCT patients with CMV infection and the effect of gB type on clinical outcome including CMV disease.

Study design: The diagnosis of HCMV infection after allogeneic HSCT was detected by Antigennium (AGM) and Nested-PCR (N-PCR). All patients with CMV infection received ganciclovir preemptive treatment. Positive samples from patients with active HCMV infection were subtyped to genotyping using the N-PCR to amplify a region of UL55, followed by restriction analysis based on Hinf I and Rsa I digestion and subsequent sequencing aligned with known CMV variants in GenBank.

Results: We evaluated 55 allogeneic HSCT recipients, 41/55 patients (74.5%) presented active HCMV infection detected by AGM and N-PCR. The N-PCR median time of 32 days after the transplant. The distribution of HCMV gB genotypes in 30/41 patients with HCMV active infection was as follows: gB1, 14/30 (46.6%); gB2, 10/30 (33.3%); gB3, 2/30 (6.7%); gB4, 2/30 (6.7%) and two patients (6.7%) had mixed infection with gB1-gB3 and gB2-gB3. The sequencing confirmed the four CMV gB genotypes. HCMV disease developed in 2 patients, characterized for gastrointestinal disease, and these two patients had infection with a mixture of HCMV gB genotypes.

Conclusions: In this study the most prevalent genotype in patients with HCMV active infection was gB type 1 and moreover, the mixture of HCMV gB genotypes was associated with gastrointestinal disease. It suggests that gB genotypes may have influence on the patient’s outcome in a Brazilian population.

RELAPSE AFTER ALLOGENEIC STEM CELL TRANSPLANTATION (SCT): RETRANSPANTATION DOES NOT IMPROVE OUTCOME IN COMPARISON TO SUPPORTIVE CARE AND CHEMOTHERAPY

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Relapse post allogeneic SCT is a life threatening event and we report here our treatment strategies.

Between 12/1994 and 6/2010 449 pts. (median age: 42 years, 16-70) underwent allogeneic SCT from an unrelated (n = 263) or a matched related (n = 186) donor for treatment of acute myeloblastic leukemia, AML (n = 277) or acute lymphocytic leukemia, ALL (n = 172). 240 pts. were transplanted in CR1, 209 pts. beyond CR1, 241 pts. were male, 208 pts. were female. Post allogeneic SCT 193/449 pts. (43 %) are alive in complete remission (CR), 142/449 pts. (32 %) relapsed and 114/449 pts. (25 %) died due to non relapse mortality (NRM). In the 142 relapsed patients the median duration of remission post allogeneic SCT was short (5 months, 1-135) and the median age of the patients at relapse was 43 years (17-68). Our treatment strategies for relapse were as follows: Firstly stop of immunosuppression. Thereafter, in fit patients reinduction chemotherapy with BuFlu or BuCy and in children after a variety of regimens. In the case of death by mixed chimerism 21/28 pts. (75 %) at a median of 4 months (1-13) post 2nd SCT. 7728 pts. (25 %) died due to NRM (gvhd, infection, toxicity) at a median of 1 month (0-15) after 2nd SCT. The time interval between relapse and 2nd SCT was in median 4 months (1-56). Survival after 1st relapse in the Re-SCT group was 10 months (3-60 months). With conventional therapy 7/112 pts. (6 %) are alive with a median follow-up of 17 months (2-50) after relapse and 105/112 pts. are dead. Causes of death are leukemia in 104/105 pts., 2nd malignancy in 1/105. Median survival after 1st relapse and conventional therapy is 2 months (0.1-50 months).

In Conclusion: Prognosis of relapse after allogeneic SCT is dismal. The longer survival of the Re-SCT group in comparison to the conventional group might reflect a selection of better risk patients. Prospective studies are needed to identify patients who will benefit from a 2nd SCT as compared to non-transplant treatment.

THE TIMING OF ALEMTUZUMAB SIGNIFICANTLY IMPACTS MIXED CHIMERISM AND ACUTE GRAFT VERSUS HOST DISEASE

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Reduced-intensity conditioning (RIC) regimens that contain alemtuzumab are often associated with a high incidence of mixed chimerism and a low incidence of acute graft versus host disease (GVHD). Both observations may be due to alemtuzumab-mediated depletion of graft lymphocytes. To determine if the timing of alemtuzumab administration affects the incidence of mixed chimerism and acute GVHD, we compared the outcomes of 102 patients with non-malignant diseases who underwent RIC-HCT using fludarabine and melphalan at our institution with 2 different alemtuzumab schedules. Forty-nine patients received a proximal alemtuzumab schedule, consisting of a median dose of 1mg/kg alemtuzumab divided over 4-5 consecutive days between days 12-4. Fifty-two patients received a distal alemtuzumab schedule, consisting of a median dose of 2.6mg/kg alemtuzumab divided over 3-4 consecutive days between days -23 to -19. All patients engrafted at a median of 11.5 days with the exception of 1 patient in each group who died at days +7 and +15. Excluding these 2 patients, the overall incidence of acute rejection and/or mixed chimerism was (2 patients with acute rejection) was 35/48 patients (73%) versus 20/51 patients (39%) in the distal group (no acute rejection) (p = 0.01). Acute GVHD prophylaxis was rapidly weaned in the majority of patients with mixed chimerism. Six patients in the proximal group and 4 patients in the distal group received hematopoietic cell boost (p = 0.16), and donor lymphocyte infusions (DLI) were administered to 18 patients in the proximal group and 5 patients in the distal group (p = 0.002). Prior to these interventions, acute GVHD grades II-IV developed in only 1 patient in the proximal group (2%), and 9 patients in the distal group (17%) (p = 0.05). Following these interventions, acute GVHD grades II-IV occurred in 7 patients in the proximal group and 6 patients in the distal group, making the total overall incidence of acute GVHD grades II-IV 16% in the proximal group versus 29% in the distal group (p = 0.1). Ultimately, 4 patients in the proximal group and 1 patient in the distal group required a second transplant. We conclude that the timing of alemtuzumab significantly influences the incidences of both mixed chimerism and acute GVHD. Further studies to optimize the dosing schedule of alemtuzumab are warranted.

NOT GETTING HIGH ON BUSULFAN: A NOVEL APPROACH TO AVOID HIGH BUSULFAN LEVELS IN ADULTS AND CHILDREN UNDERGOING HSCT

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Between 12/1994 and 6/2010 449 pts. (median age: 42 years, 16-70) underwent allogeneic SCT from an unrelated (n = 263) or a matched related (n = 186) donor for treatment of acute myeloblastic leukemia, AML (n = 277) or acute lymphocytic leukemia, ALL (n = 172). 240 pts. were transplanted in CR1, 209 pts. beyond CR1, 241 pts. were male, 208 pts. were female. Post allogeneic SCT 193/449 pts. (43 %) are alive in complete remission (CR), 142/449 pts. (32 %) relapsed and 114/449 pts. (25 %) died due to non relapse mortality (NRM). In the 142 relapsed patients the median duration of remission post allogeneic SCT was short (5 months, 1-135) and the median age of the patients at relapse was 43 years (17-68). Our treatment strategies for relapse were as follows: Firstly stop of immunosuppression. Thereafter, in fit patients reinduction chemotherapy with BuFlu or BuCy and in children after a variety of regimens. In the case of death by mixed chimerism 21/28 pts. (75 %) at a median of 4 months (1-13) post 2nd SCT. 7728 pts. (25 %) died due to NRM (gvhd, infection, toxicity) at a median of 1 month (0-15) after 2nd SCT. The time interval between relapse and 2nd SCT was in median 4 months (1-56). Survival after 1st relapse in the Re-SCT group was 10 months (3-60 months). With conventional therapy 7/112 pts. (6 %) are alive with a median follow-up of 17 months (2-50) after relapse and 105/112 pts. are dead. Causes of death are leukemia in 104/105 pts., 2nd malignancy in 1/105. Median survival after 1st relapse and conventional therapy is 2 months (0.1-50 months).

In Conclusion: Prognosis of relapse after allogeneic SCT is dismal. The longer survival of the Re-SCT group in comparison to the conventional group might reflect a selection of better risk patients. Prospective studies are needed to identify patients who will benefit from a 2nd SCT as compared to non-transplant treatment.
of regimens. Even with therapeutic drug monitoring of Bu, by the
time results come back, patients may have had 1 or 2 days of high
or low exposure. The required dose change to target a desired cumu-
latice AUC may exceed the clinicians’ willingness to make such a
change. We report here our use of a novel dosing schedule which
always avoids high exposure and is compatible with samples being
sent across states for transplant-related assays.

Methods: 96 patients from 6 institutions were given Bu over 5 days.
Regimens included BuCy(n = 36), BuFlu(n = 24). Four blood sam-
ples were collected after infusion of a half dose on day 1 and trans-
ported to CHW. On day 2 the second half dose was given whilst
Bu measurement and pharmacokinetic analysis was completed. A
full dose AUC was predicted from the half dose results. On days 3
to 5 patients were administered the remaining Bu doses, modified
where necessary based on the day 1 AUC. In 71 patients, repeat
AUC analysis after a full dose allowed comparison of predicted
with actual AUC.

Results: The day 1 analysis predicted a full dose AUC range between
3148 and 12666 μM.min. Thirty four patients would have had an
AUC > 6000 μM.min. For days 3 to 5, 38 had doses reduced and
10 had doses increased to target a desired AUC. After dose adjust-
ments, the repeat AUC was within ±10% predicted in 38 and within
±15% in 51. Two of 36 adults had a final AUC value > 6000 μM.min
(but only 6097 and 6187).

Conclusions: There was a wide variability in Bu exposure following
the first dose: 35% of the patients were predicted to be exposed to
levels above the recommended level of 6000 μM.min. With dose ad-
justment, targeted dose exposure was within ±15% of predicted
values in 72% of patients and no adult was exposed to an AUC sig-
ificantly > 6000 μM.min. This novel dosing schedule allows real-
time pharmacokinetic dose adjustment in multiple centres across
Australia and avoids the problem of having to catch up, or back off
on day 3 and 4 dosing. It is simple and lends itself to centres that
are remote from the lab testing the Bu levels. The impact of concom-
itant medication on the remaining variability in exposure continues
to be studied.

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PILOT STUDY ON THE MEASUREMENT OF CALCINEURIN PHOSPHATASE ACTIVITY ON DAY 21 IN ALLOGENEIC STEM CELL RECIPIENTS
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Background: Tacrolimus (TAC) suppress T-cell activation by the
action on calcineurin (CN). CN activity was assessed in the allogene-
ic recipients who were treated with TAC for graft-versus-host dis-
ase (GVHD) prophylaxis to investigate whether CN activity increased in patients with severe acute GVHD.

Methods: Forty patients who underwent allogeneic stem cell trans-
plantation (SCT) using TAC as GVHD prophylaxis were analyzed for GVHD. Among them, CN activity was analyzed in the 10 con-
secutive patients. TAC was administered at a dose of 0.03 mg/kg in-
travenously from day-1 to +21. TAC levels and CN activity were
assessed on day-1 before TAC administration and days 0, +3, +7,
+14, and +21. Target TAC concentration (15-20 ng/ml) was main-
tained during the current study.

Results: The cumulative incidence of acute GVHD (74.1% vs.
60.3%, p = 0.888) and severe chronic GVHD (22.5% vs. 33.3%, p
= 0.539) were not different between groups with high and low
TAC trough levels. CN activity on day-1 was 0.12±0.09 nmol and
had decreased from baseline level (0.29±0.15 nmol) (p < 0.001).
There was no correlation between CN activity and TAC concen-
trations (r = 0.024). CN activity was steady-state during post-trans-
plant days 0 to +14 regardless of the GVHD. CN activity on day-21 for those with grade 2-4 acute GVHD showed a higher
CN activity (0.18±0.04 nmol) compared to those without grade 2-
4 acute GVHD (0.14±0.05 nmol, p = 0.462). The cumulative inci-
dence of acute GVHD (40% vs. 80%, p = 0.248) and chronic
GVHD (20% vs. 70%, p = 0.464) between low and high CN activity
and were not significantly different.

Conclusion: Though GVHD was higher for the high CN activity
patient group, this pilot study failed to demonstrate significant difference
due to small sample size. However, the patients manifesting
GVHD with high CN activity on post-transplant D+21 may need
to be treated with other kinds of immunosuppressive agent regard-
less of drug level.

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CHARACTERIZATION OF ORAL INVOLVEMENT IN ACUTE-GRAFT-VERSUS-
HOST DISEASE
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Introduction: Acute graft-versus-host disease (aGVHD) is a major
complication of allogeneic hematopoietic cell transplantation (HCT).
The purpose of this study was to characterize the oral features associated with aGVHD.

Methods: Patients that underwent allogeneic HCT at Dana-Farber/
Brigham and Women’s Center (Boston, MA) between 1995 and
2010 and developed prominent oral aGVHD were identified. Data
was collected from patient medical records using a standardized
form and analyzed descriptively.

Results: Eighteen cases were identified, of which 5 (28%) only dem-
onstrated oral features; the remaining 13 had variable involvement of
skin (13/18, 72%), liver (6/18, 33%), and gut (5/18, 28%). Oral mu-
cositis preceded aGVHD in 10 (56%) patients. The median time to
onset of oral aGVHD was 34 days (range 11-139). Intraoral sites af-
fected by non-specific ulcerations included the tongue (16/18, 89%);
dorsum in 7/18), bucal mucosa (16/18, 89%), labial mucosa (13/18,
72%), palate (12/18, 67%; hard palate in 7/18), and floor of mouth
(6/18; 33%); 7 (39%) cases presented with prominent lip ulceration
and crusting. Salivary gland disease features included severe hypo-
function (1/18, 6%) and palatal mucocleae (1/18, 6%). In addition
to systemic therapies, topical preparations of dexamethasone (10/
18; 56%), tacrolimus (7/18; 39%), and morphine (3/18; 17%) were
utilized for ancillary support. Of the 13 (72%) patients that survived
day beyond day100, two developed oral aGVHD.

Conclusions: Oral features of aGVHD include extensive non-spe-
cific ulcerations of keratinized and non-keratinized mucosa and are
often observed in the context of concurrent skin, liver and gut in-
volvement. Intensive topical therapies may be helpful in reducing
symptoms and promoting healing. Concurrent salivary gland
involvement appears to be infrequent.

Relevance: Oral medicine specialists can play an important role in
both its diagnosis and management; the HCT team should be aware
that aGVHD can present with oral features that might be responsive
to topical therapies.

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SCREENING FOR HEMOGLOBINOPATHIES IN ALLOGENEIC CORD BLOOD
UNITS USING CAPILLARY ELECTROPHORESIS
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Introduction: Hemoglobinopathies are common hereditary dis-
ases with very different clinical outcomes. Since severe hemoglobin-
opathies like sickle cell anemia or thalassemia major are by
themselves indications for transplantation, these inborn errors are
relevant screening parameters for transplantation centers. In line
with that, the international FACT-Netcord standards for the storage
and release of cord blood units for transplantation (4th edition) state
that screening for homozygous hemoglobinopathies is mandatory
for cord blood banks storing allogeneic transplants.

Material and Methods: We use the Capillarys 2 hemoglobin elec-
trophoresis machine and the Capillaries cord blood procedure by Se-
bia. Analyses are performed on anti-coagulated cord blood before
volume reduction. The red blood cells are sedimented and plasma
is removed completely. Before measuring the samples are hemolysed
automatically.

Results: So far, a total of 717 measurements were performed. In
1.5% of all samples hemoglobin variants were detected, namely six
cases of Hb Barts, three cases of heterozygous Hb S and one case
of a heterozygous hemoglobin variant, most likely Hb E. These sam-
ples were then sent to a specialized laboratory for PCR-testing, and