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Antibiotic exposure prior to respiratory viral infection is associated with progression to lower respiratory tract disease in allogeneic hematopoietic cell transplant recipients

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Abstract

Introduction: Recent publications note an association between antibiotic exposure and respiratory viral infections (RVIs). Antibiotics affect microbiota and impair immune response against RVIs in mice, and low microbiome diversity is associated with pulmonary complications including viral lower respiratory tract disease (LRTD) in hematopoietic cell transplantation (HCT) recipients. In this study, we examined whether antibiotic exposure was associated with increased risk of disease progression in RVIs post-transplantation.

Materials and Methods: We analyzed patients who underwent allogeneic HCT (6/2008-2/2016) and had their first RVI due to parainfluenza virus (PIV), respiratory syncytial virus (RSV) or human metapneumovirus (MPV) during the initial 100 days post-transplantation. Antibiotic exposure in the three weeks before RVI onset was defined as *(i)* use of specific

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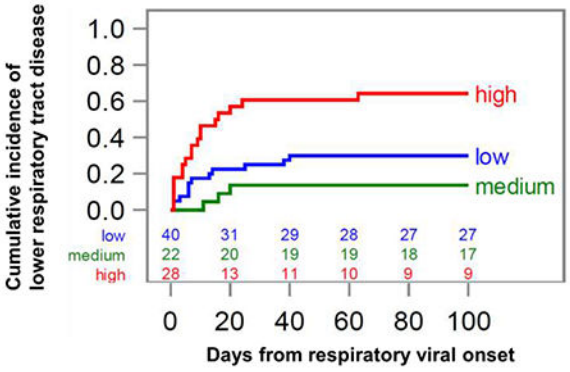
antibiotics versus none of these antibiotics, and *(ii)* number of antibiotic-days. Cox proportional hazards models were used to examine associations between antibiotic exposures and risk of viral disease progression to proven/probable/possible LRTD.

Results: Ninety HCT recipients (84 adults, 6 children) fulfilled study criteria; 33 progressed to LRTD. The number of antibiotic-days was associated with progression to LRTD after adjusting for neutropenia, steroid use, and either lymphopenia (hazard ratio, 1.41 [95% confidence interval (CI) 1.04–1.92], P=.027) or monocytopenia (hazard ratio, 1.46 [95% CI, 1.11–1.91], P=.006). Specific antibiotic classes was not associated with the outcome.

Conclusions: Cumulative antibiotic exposure immediately before RVI onset is a risk factor for disease progression following PIV, RSV and MPV infections post-transplantation. Larger cohort studies are needed to determine the impact of specific antibiotics/antibiotic classes on disease severity.

Graphical Abstract

Cumulative incidence of lower respiratory tract disease stratified by antibiotic exposure before respiratory viral infection



Keywords

respiratory virus; antibiotics; microbiota; lower respiratory tract disease; hematopoietic cell transplantation; progression

Introduction

Prophylactic and empiric antibiotic use are part of standard care for high-risk patients, especially hematopoietic cell transplant (HCT) recipients, and reduce the risk of morbidity and mortality associated with life-threatening bacterial infections.[1–4] On the other hand, emerging data suggest that antibiotic exposure can affect other adverse transplant outcomes.[5–7] Although the exact mechanism remains elusive, the immunomodulatory effects of antibiotics via altered gut microbiota on the susceptibility to bacterial infection or graft-versus-host disease (GVHD) may be a plausible explanation.[6, 8–13]

Recent animal and human data demonstrate the intriguing link between antibiotic exposure, microbiota and respiratory viral infections (RVIs). Studies in mice show a relationship

between disrupted microbiota and impaired immune control of RVI in an antibiotic-treated mouse model, and the protective effect of *Lactobacillus* priming against lethal pneumonia virus.[14–18] Also, human studies show reduced respiratory disease severity with probiotic use in immunocompetent populations, and the association between low microbiome diversity and pulmonary complications including viral pneumonia in HCT recipients.[19–22] RVIs are frequently associated with lower respiratory tract disease (LRTD) and mortality in HCT recipients.[23–25] Progression to LRTD may also be affected by antibiotic exposure; however, little clinical data exist to support this hypothesis.

The objective of this study was to investigate the effect of antibiotic use on respiratory disease progression following RVIs in transplant recipients. We hypothesized that either pre- or post-transplant antibiotic exposure would increase the risk of disease progression of RVIs occurring after allogeneic HCT.

Materials and Methods

Study Design

We retrospectively reviewed allogeneic HCT recipients whose first RVIs were diagnosed by multiplex respiratory viral PCR at the Fred Hutch in the first 100 days post-transplant. We selected patients with parainfluenza virus (PIV), respiratory syncytial virus (RSV) or human metapneumovirus (MPV) detected from their respiratory tracts without respiratory copathogens. These viruses were selected given the well-established respiratory pathogenesis in this population. Influenza virus was not evaluated due to limited number of cases for the current study.[26] Consecutive subjects were identified from two cohorts. The first cohort included patients who underwent transplantation from March, 2010 through February, 2016, and had nasal samples collected and tested for clinical purposes when respiratory symptoms were present. The second cohort was a subset of a prospective surveillance study of HCT recipients undergoing transplantation from June 2008 through February 2010 where standardized respiratory symptom surveys and multiplex respiratory PCR tests were performed weekly during the first 100 days post-transplant.[27] For this analysis, we included subjects with respiratory symptoms at the time of first detection of respiratory viruses and those with complete blood cell counts measured within two weeks before the virus detection. Demographic and clinical data were extracted from the Fred Hutch's database and medical chart review. The study was approved by the Institutional Review Board at the Fred Hutch.

Laboratory Testing and Definitions

Reverse transcription PCR was performed for 12 respiratory viruses on respiratory samples, as described previously.[28, 29] Proven or probable LRTD (proven/probable LRTD) was defined as having virus detected by PCR from a lower respiratory tract sample (e.g., bronchoalveolar lavage) with or without new pulmonary infiltrates by chest radiography, respectively. Possible LRTD was defined as having virus detected by PCR from an upper respiratory tract sample with new pulmonary infiltrates.[30] To measure the severity of patients' underlying conditions, the HCT comorbidity index was used. The HCT comorbidity index is comprised of 17 different categories of organ dysfunction and is an

established tool to predict nonrelapse and overall mortality after HCT.[31, 32] HCT comorbidity index scores ≥3 were categorized as high risk and scores 0-2 were considered low risk for poor transplant outcomes.

The date of the first detection of one of the three respiratory viruses from the upper respiratory tract sample was considered to be date of viral onset and served as time zero for the progression to LRTD outcome. For patients diagnosed with LRTD on the same date as the first detection of virus, time zero was assigned to be one day before that date. Analysis time was defined as the number of days from time zero to LRTD, death, or day 100 following viral onset, whichever came first.

Antibiotic use prior to viral onset was the main baseline exposure. We defined four different time windows for antibiotic exposures before viral onset: (1) Pre-transplantation period (Day -21 to Day -1 of transplantation), (2) Immediate post-transplantation period (Day 0 to Day +21 of transplantation or to one day prior to viral onset, whichever came first), (3) Immediate pre-onset period (three weeks prior to one day before viral onset), and (4) Pre- and post-transplantation period (Day -21 of transplantation to one day prior to viral onset) (Supplementary Figure 1). Antibiotic exposure for each window was defined as (i) use of specific antibiotics versus none of these antibiotics, and (ii) average number of antibiotics per day. The average number of antibiotics per day was computed for each patient by dividing the cumulative sum of the number of antibiotics received during each day in the time window (total antibiotic-days) by the number of days in the window.

Antimicrobials against *Pneumocystis jiroveci pneumonia* and fungus were excluded except for trimethoprim/sulfamethoxazole. Prophylactic use of trimethoprim/sulfamethoxazole typically continues until two days prior to transplantation and resumes after engraftment post-transplantation. Prophylactic use of trimethoprim/sulfamethoxazole was counted as a daily regimen pre-transplantation and as a twice-weekly regimen post-transplantation according to our institutional standard practice. Antibiotic prophylaxis during neutropenia (levofloxacin for adults and ceftazidime for children) is our standard practice, beginning at the onset of neutropenia and continuing until engraftment.

In addition to assessing individual antibiotics (trimethoprim/sulfamethoxazole and intravenous vancomycin), some antibiotics were grouped based on the specific antimicrobial activity of our interest as follows: (1) Antibiotics with significant anaerobic activity (meropenem, imipenem, ertapenem, piperacillin/tazobactam, ampicillin/sulbactam, amoxicillin/clavulanate, metronidazole, moxifloxacin or clindamycin), (2) Antibiotics with significant gram positive activity (vancomycin, daptomycin, linezolid, or clindamycin), (3) ciprofloxacin or levofloxacin, (4) carbapenems (meropenem, imipenem, or ertapenem), and (5) antipseudomonal cephalosporins (ceftazidime or cefepime).

Statistical Analysis

We estimated the cumulative incidence of respiratory viral disease progression to LRTD, treating death as a competing risk. After comparing the cumulative incidence curves by baseline antibiotic exposures for each of the above-mentioned time windows, we selected one window to estimate the association between antibiotic exposure and progression to

LRTD in unadjusted and adjusted Cox proportional hazards models. Proven/probable LRTD and proven/probable/possible LRTD were analyzed as separate outcomes, in separate models. Baseline covariates for potential confounders were selected from previously-identified risk factors for disease progression, important biological variables, and factors thought to influence antibiotic use, such as neutropenia during the window in which antibiotic exposure was measured.[23, 24, 30, 33–35] These covariates were then tested for associations with LRTD using univariable Cox models. Novel GVHD prophylactic strategies including T cell depletion, alemtuzumab and abatacept were not our standard practice and not analyzed in this study. Due to the limited number of events, multivariable models were considered only for the combined proven/probable/possible LRTD outcome and included a maximum of four variables. Each antibiotic exposure (overall, individual antibiotics, specific groups of antibiotics) was analyzed in separate models, using the same approach as outlined below. To guide selection of confounders of the association between antibiotic exposure and progression to LRTD, we proposed two directed acyclic graphs (DAGs). We used the set of minimal sufficient adjustment variables identified by each DAG to select covariates for inclusion in multivariable models.[36, 37] Due to the high correlation between lymphopenia and monocytopenia at viral onset, we included these variables in separate multivariable models. Because our selected time window had the same number of days for all patients, estimates for average number of antibiotics per day were reported as hazard ratios for a 7-unit increase in the number of antibiotic-days, for easier interpretation. The proportional hazards assumption was assessed by graphical checks based on martingale residuals and by testing for an interaction between the logarithm of analysis time and each covariate. To address any concerns of antibiotic use for rapidly progressing respiratory illness immediately prior to our defined date of viral onset, we performed sensitivity analyses in which we shifted time windows to end three days prior to viral onset rather than one day prior to onset. Two-sided *P* values <.05 were considered statistically significant. All statistical analyses were performed using SAS 9.4 for Windows (SAS Institute, Inc., Cary, NC).

Results

Patient Characteristics

We identified 84 adult and 6 pediatric HCT recipients fulfilling the study criteria (PIV = 51, RSV = 23, MPV = 16) (Table 1). Time to viral onset following HCT was evenly distributed in the first 100 days (median 51 days, interquartile range 27-67 days). Relatively high HCT comorbidity index scores (58% with scores ≥3) and steroid use (51%) were common in this cohort. Relapse of the underlying malignancy occurred in two patients before the viral onset; neither received chemotherapy for the relapse.

Antibiotic use

All patients received at least one antibiotic in the pre- and post-transplantation period before viral onset. The numbers of patients who did not receive any antibiotics for the other windows are as follows: 4 in the pre-transplantation period, 1 in the immediate post-transplantation period, and 7 in the immediate pre-onset period. Supplementary Figure 2 shows the distribution of the average number of antibiotics per day for each patient in the

four specified time windows; the medians were 1.0 (pre-transplantation period), 1.0 (immediate post-transplantation period), 0.4 (immediate pre-onset period) and 0.8 (pre- and post-transplantation period).

Trimethoprim/sulfamethoxazole, levofloxacin, and ceftazidime were most commonly used in all windows but immediate post-transplantation period, where trimethoprim/sulfamethoxazole was rarely used, as expected based on our standard clinical protocol (Supplementary Figure 3). The distribution of the average number of antibiotics per day according to individual antibiotics or grouped antibiotics of interest was compared in each window (Supplementary Figure 4). All antibiotics of interest other than trimethoprim/sulfamethoxazole were most frequently administered in immediate post-transplantation period.

Cumulative incidence of LRTD according to prior antibiotic exposure

Of the 90 patients, 33 progressed to proven/probable/possible LRTD (12 proven/probable LRTD). Among the 33 patients who progressed to LRTD during observation, the median number of days from onset to progression was 7 days (interquartile range 4- 16 days). Six patients were diagnosed with LRTD on the date of first detection: four of these were diagnosed with possible LRTD. Figure 1 shows the cumulative incidence of proven/probable/possible LRTD by tertiles of the overall average number of antibiotics per day in each window. The largest separation of curves between the high antibiotic exposure group and the lower exposure groups was observed in the immediate pre-onset period (three weeks prior to one day before viral onset). Using this window, we noted a similar pattern in the cumulative incidence curves for several groups of antibiotics or individual antibiotics (Supplementary Figure 5), with these trends being more prominent than those seen in other windows considered (data not shown). Based on these descriptive curves, we chose the immediate pre-onset period to estimate the effect of antibiotic exposure on the LRTD outcome using Cox proportional hazards models.

Univariable and multivariable model estimates for association with progression to LRTD

Univariable model estimates for association with progression to proven/probable LRTD and to proven/probable/possible LRTD are shown in Figure 2. Cumulative antibiotic exposure (number of antibiotic-days) and exposure to ciprofloxacin/levofloxacin were associated with increased risk of disease progression in both models. Overall, the trends of hazard ratios were similar between proven/probable/possible LRTD and proven/probable LRTD alone as outcomes; the hazard ratios for proven/probable LRTD alone were higher in some specific antibiotic groups (e.g., vancomycin/linezolid/daptomycin) than those for proven/probable/possible LRTD. In addition to antibiotic exposure, leukopenia (lymphopenia, monocytopenia and neutropenia) at or in the three weeks prior to viral onset was associated with increased risk of disease progression. In contrast, no factors measured pre-transplantation (e.g., age, conditioning regimen, leukopenia) were found to be significant. Viral onset in the first 30 days post-transplant was significantly associated with higher risk of proven/probable/possible LRTD outcome, which was thought to be mainly driven by leukopenia immediately after HCT.

Based on our first proposed DAG (Figure 3a), we evaluated multivariable models adjusting for lymphopenia at viral onset, neutropenia frequency in the three weeks prior to onset, and steroid use in the two weeks prior to onset. We also ran similar models that included monocytopenia at onset instead of lymphopenia. Both sets of models provided similar results (Figure 4). Cumulative antibiotic exposure remained significantly associated with progression to proven/probable/possible LRTD, with hazard ratios very similar to the unadjusted estimates. For antibiotics with significant anaerobic activity, carbapenems, and ciprofloxacin/levofloxacin, adjusted estimates for associations with progression to LRTD were no longer statistically significant.

Our second proposed DAG was similar to our first DAG, but added the assumption that patients with more complicated clinical status (as measured by the HCT comorbidity index) are not only more likely to receive antibiotics, but more likely to progress to LRTD (Figure 3b). The set of adjustment variables identified for this DAG included the same set as listed for our previous models, but with the addition of HCT comorbidity index. Because this led to more than the maximum number of variables we could include given our limited number of events, we evaluated bivariable models including HCT comorbidity index and each antibiotic exposure variable. The antibiotic estimates did not change substantially (data not shown), indicating the HCT comorbidity index did not have a substantial confounding effect on the association between antibiotic exposure and progression to LRTD. Separately, we performed sensitivity analyses using a modified immediate pre-onset period, in the three weeks through three days prior to viral onset; the results remained similar (Supplementary Figure 6).

Discussion

Our results support the hypothesis that prior antibiotic exposure may impact the disease severity of respiratory viruses in allogeneic transplant recipients. Cumulative antibiotic exposure immediately prior to the onset of PIV, RSV and MPV upper respiratory infection was associated with increased risk of progression to LRTD in adjusted models. Exposures to specific antibiotics were not significantly associated with increased risk of progression.

Recent randomized controlled trials indicate that even short-term antibiotic use may lead to significant changes in the gut microbiota.[38, 39] Antibiotic exposure and low microbiome diversity have been increasingly recognized as important risk factors for poor transplant outcomes including GVHD and bacterial infection.[3, 6, 8–13, 40] The potential adverse impact of antibiotic exposure on transplant outcomes is further highlighted by a recent randomized trial in which azithromycin prophylaxis given for two years post-transplantation resulted in worse airflow decline-free survival and higher rates of hematologic relapse when compared to placebo.[7] Altered microbiota induced by antibiotic use may play a role in immune modification and transplant outcomes.[6, 8–13] Interestingly, rates of disease progression to LRTD vary widely from 5% to 55% for PIV, RSV and MPV, depending on surveillance measures and transplant centers.[23, 24, 41] These findings raise the question of whether different antibiotic practices between transplant centers may be an important reason for the observed differences in progression rates. The epidemiological link observed in the current study may provide rationale to further probe the impact of microbiota on transplant

outcomes including respiratory viral disease progression. This premise is consistent with recently published data showing that the abundance of butyrate-producing bacteria is negatively correlated with respiratory viral disease progression.[42] When transplant outcomes are evaluated in future studies of antimicrobial deescalation strategies, different antibiotic utilization strategies or other strategies modifying the microbiome, respiratory disease progression could be assessed as an important endpoint.

With the guide of cumulative incidence curves for LRTD, we chose the immediate preonset period to estimate the effect of antibiotic exposure on the LRTD outcome. Our results may imply that recent antibiotic exposure is more likely to impact respiratory disease progression than distant antibiotic exposure. In fact, mice showed diminished immune responses against influenza virus when inoculated immediately after the course of antibiotics.[14, 16] It is important to note that the magnitude of our reported association between overall antibiotic exposure and LRTD (adjusted HR of 1.5) represents the increased risk associated with a 7-day increase, to match a short course of antibiotics. Longer or concurrent antibiotic courses would be associated with higher risks. For example, the estimated HR for each unit increase in the average number of antibiotics per day would be nearly 2.8. We chose to examine associations of some antibiotic classes based on spectrum of antimicrobial activity because this might provide deeper insight into mechanisms of action. For example, antibiotics with activity against *Clostridiales* (grampositive anaerobic bacteria) are associated with increased risk of GVHDor transplant-related mortality; the use of *Clostridiales*-sparing antibiotics has been proposed.[5, 8] The current study did not demonstrate a statistically significant association between use of specific antibiotic classes and progression to proven/probable/possible LRTD in adjusted models, although a trend was observed between the use of certain antibiotic groups (e.g., antibiotics with significant anaerobic activity or carbapenems) and an increased risk of progression.

We adjusted for potential confounders based on DAGs that included previously identified risk factors for disease progression, important biological variables, and factors presumably related to antibiotic use.[23, 24, 30, 33–35, 37] In our case, DAGs illustrated the importance of leukopenia and steroid use as confounding variables likely to be associated with both our exposure (antibiotic use) and outcome (disease progression). Due to our small number of events, we were unable to adjust for the full set of variables identified in our second proposed DAG, where we tried to address concerns that sicker patients with more comorbidities may be more likely to be prescribed antibiotics and progress to LRTD. However, bivariable models including HCT comorbidity index and the antibiotic exposures did not show evidence for confounding effects from HCT comorbidity index. Therefore, we think it is unlikely that a full multivariable model including HCT comorbidity index would give substantially different results from the adjusted estimates we presented.

In the current study, six patients were diagnosed with LRTD on the date of first detection of respiratory viruses. It is possible that antibiotics were actually given in the context of rapidly progressive respiratory illness despite our definition that all relevant windows of interest for antibiotic exposures ended at least one day prior to viral onset. Therefore, we also performed sensitivity analyses using a modified immediate pre-onset period, in the three weeks through three days prior to viral onset and the results remained consistent.

The main limitation of this study is a relatively small sample size at a single transplant center. Despite our attempt to combine proven/probable LRTD and possible LRTD to perform adjusted model analysis, it is possible that the study was underpowered to adequately evaluate the impact of any specific antibiotic class. Although possible LRTD has been used as "LRTD" in numerous studies,[26, 43–46] it would be ideal to apply more strict criteria to increase the certainty of microbiological diagnosis for LRTD.[30] In our study, the hazard ratios for proven/probable LRTD alone were higher in the groups with exposure to some antibiotics (e.g., vancomycin, linezolid, daptomycin) than those for proven/probable/possible LRTD. If similar adjusted models for the outcome of proven/probable LRTD alone were performed among a larger cohort of patients/events, we might see larger effects of these antibiotics. Furthermore, we were unable to examine dose response relationships with some specific antibiotic classes and to determine whether our observations differ between individual viruses. Finally, despite our efforts to adjust for confounders using DAGs, it is possible other factors or different causal diagrams may explain the relationship between antibiotic exposure and LRTD; unrecognized confounders cannot be completely eliminated due to the nature of the retrospective study.

This is the first study to demonstrate that cumulative antibiotic exposure immediately prior to viral onset appears to be an important risk factor for the disease progression for some respiratory viruses in transplant recipients. Larger cohort studies are needed to determine whether exposure to specific antibiotic classes increases the risk of disease progression and whether there are differences between respiratory viruses. Ultimately, randomized clinical trials of different antibiotic utilization strategies or other strategies modifying the microbiome are needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflict of Interest statement. Michael Boeckh received research support and served as a consultant for Gilead Sciences, Merck, Ansun Bioscience, and Aviragen Therapeutics; and as consultant for Humabs Biomed. Janet A. Englund received research support from GlaxoSmithKline, Gilead, Pfizer, and Chimerix, and as a consultant for Pfizer and GlaxoSmith Kline (Data Safety Monitoring Board). Steven Pergam has served as consultant for Merck and Cubist/Optimer. All other authors declare no relevant conflict of interest.

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Highlights

- We studied effect of antibiotic exposure on respiratory virus disease post-transplant.
- Antibiotic exposure pre- and post-transplant was common before viral onset.
- Cumulative antibiotic exposure was a risk factor for respiratory disease progression.

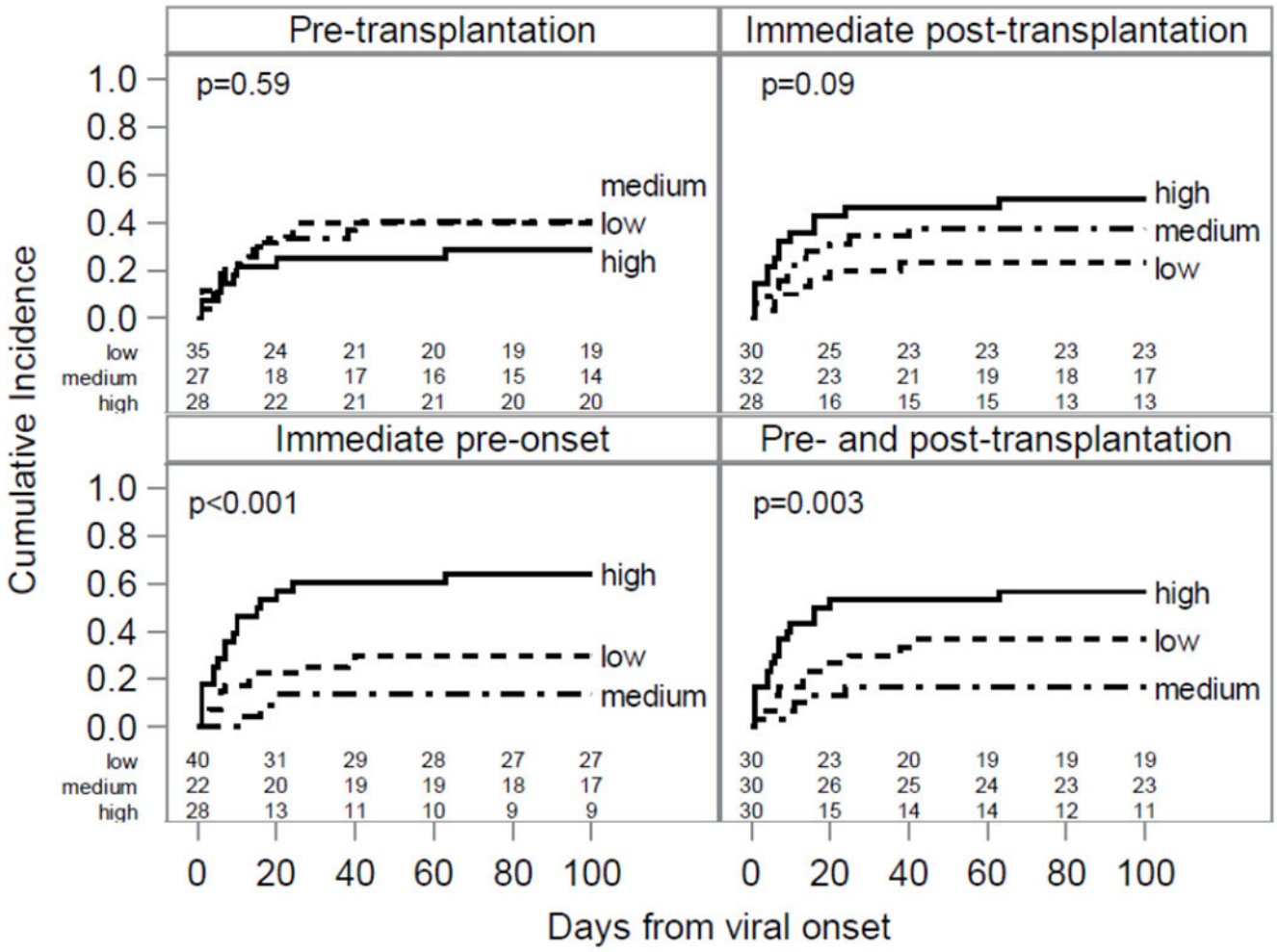


Figure 1. Cumulative incidence of proven/probable/possible lower respiratory tract disease by tertiles of overall average number of antibiotics used per day, for each time window.

Legend: Numbers above x-axis show the number of patients at risk for each group. Time windows for antibiotic exposures are as follows: (1) Pre-transplantation period (Day -21 to Day -1 of transplantation), (2) Immediate post-transplantation period (Day 0 to Day +21 of transplantation or to one day prior to viral onset, whichever came first), (3) Immediate pre-onset period (three weeks prior to one day before viral onset), and (4) Pre- and post-transplantation period (Day -21 of transplantation to one day prior to viral onset). The p-values are based on Gray's test.

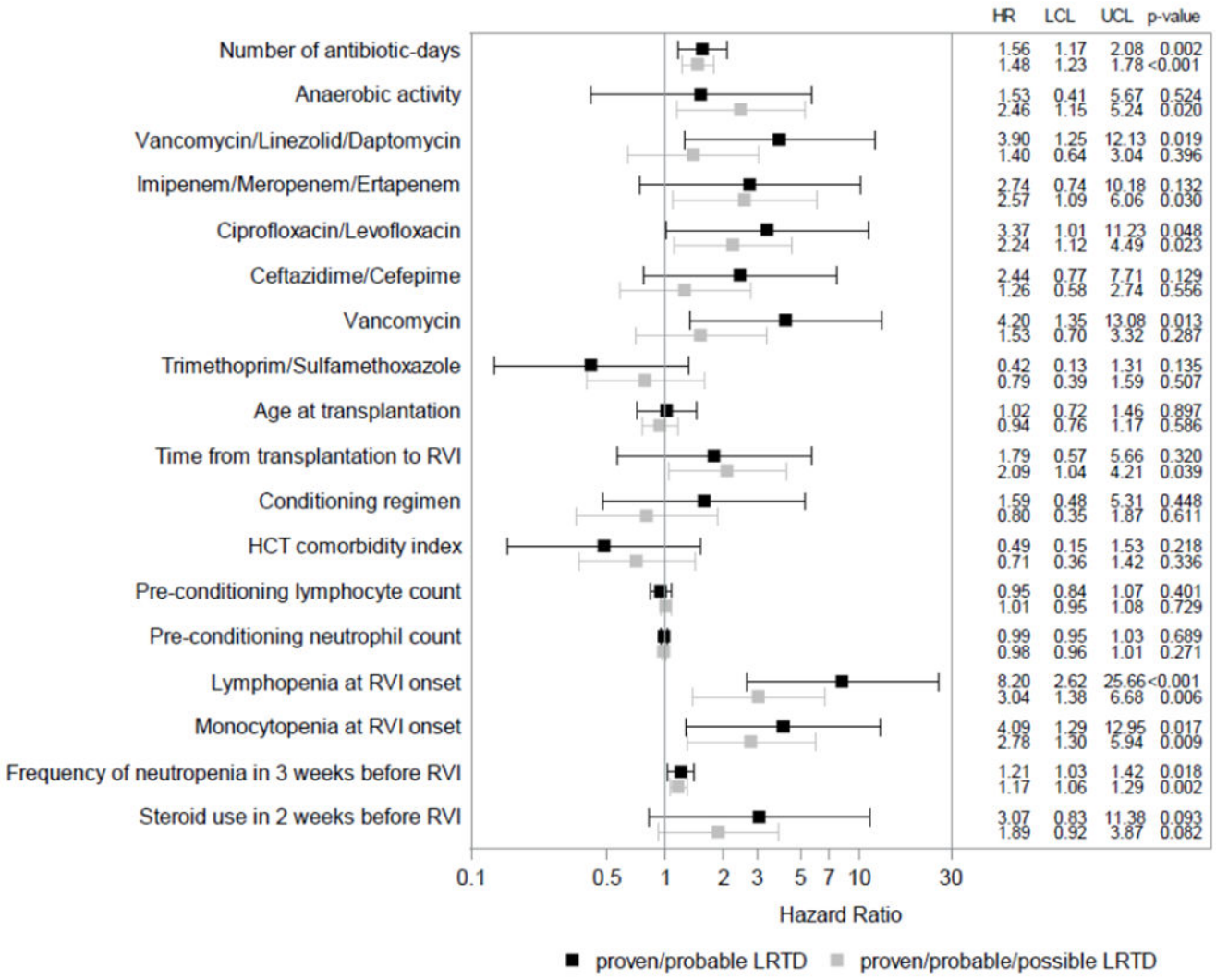


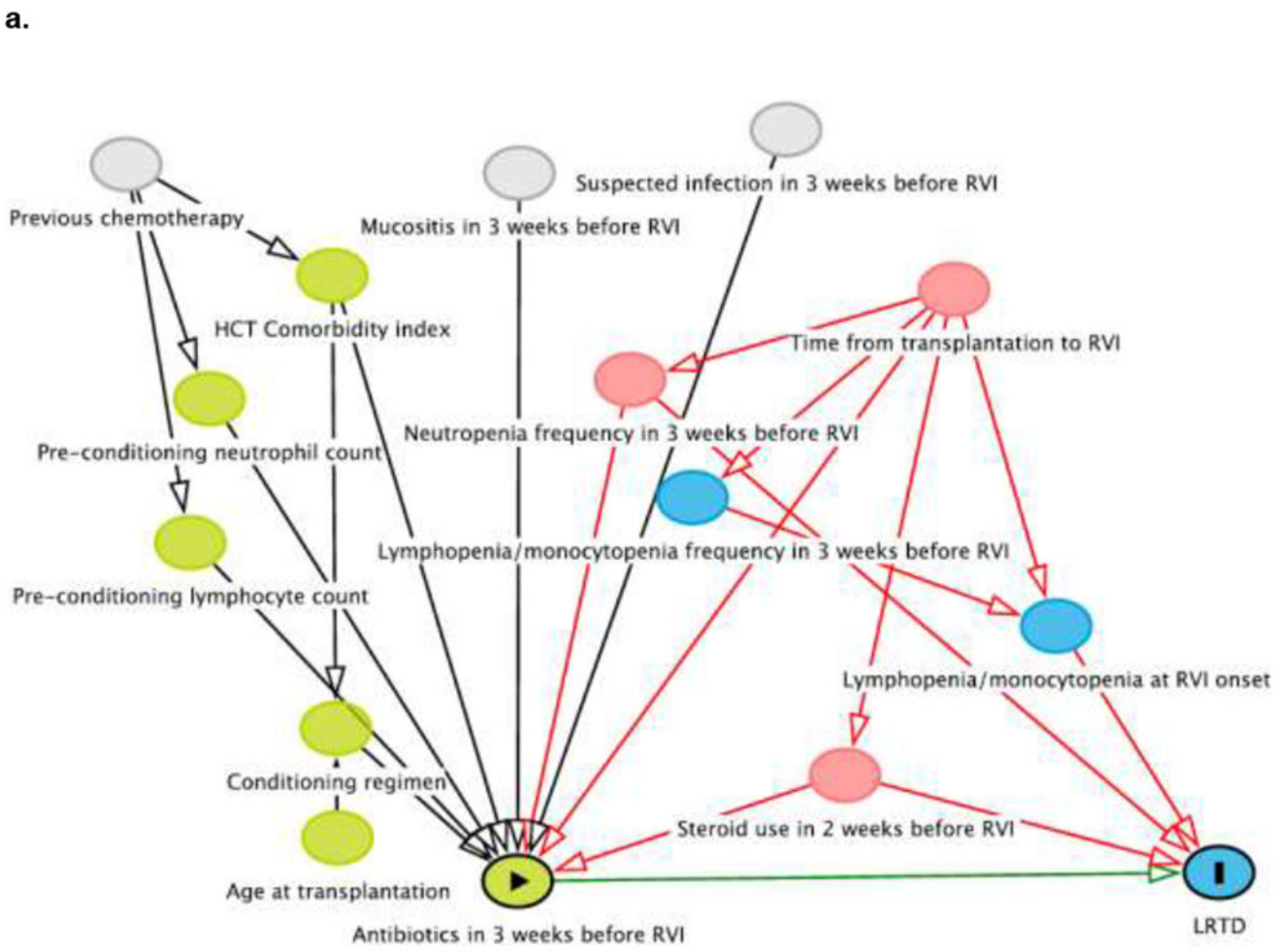
Figure 2. Univariable model estimates for associations with progression to proven/probable/ possible lower respiratory tract disease

Legend: Abbreviations: HCT, hematopoietic cell transplantation; RVI, respiratory viral infection; LRTD, lower respiratory tract disease.

Filled squares represent hazard ratios (HR) and bars represent 95% confidence intervals (LCL = lower confidence limit; UCL = upper confidence limit). Estimates shown in black represent the unadjusted estimates for associations with progression to proven/probable LRTD (12 proven/probable LRTD events). Estimates shown in grey represent the unadjusted estimates for associations with progression to proven/probable/possible LRTD (33 proven/ probable/possible LRTD events).

Antibiotic exposures in the three weeks prior to one day before viral onset were used. No patients received clindamycin in this time window. The HR for number of antibiotic-days represents the effect per 7-day increase; all other estimates represent any versus no use of the specified antibiotics. Antibiotics with anaerobic activity include meropenem, imipenem, ertapenem, piperacillin/tazobactam, ampicillin/sulbactam, amoxicillin/clavulanate, metronidazole, moxifloxacin and clindamycin. The HR for age at transplantation represents

the effect per 10 years older. Estimates for conditioning regimen compare non-myeloablative to myeloablative regimens and those for HCT comorbidity index compare values of ≥3 to 0-2. Pre-conditioning lymphocyte/neutrophil counts are the minimum value from 21 to 8 days before transplantation and HRs represent the effect per 100-unit increase. Estimates for time from transplantation compare ≤30 days to > 30 days to RVI onset. Lymphopenia/monocytopenia at RVI onset was defined as nearest count within two weeks before viral onset being < 100/mm^3. Neutropenia was defined as < 500/mm^3; HRs for frequency of neutropenia represent the effect per 10% increase in the percentage of days with neutropenia.



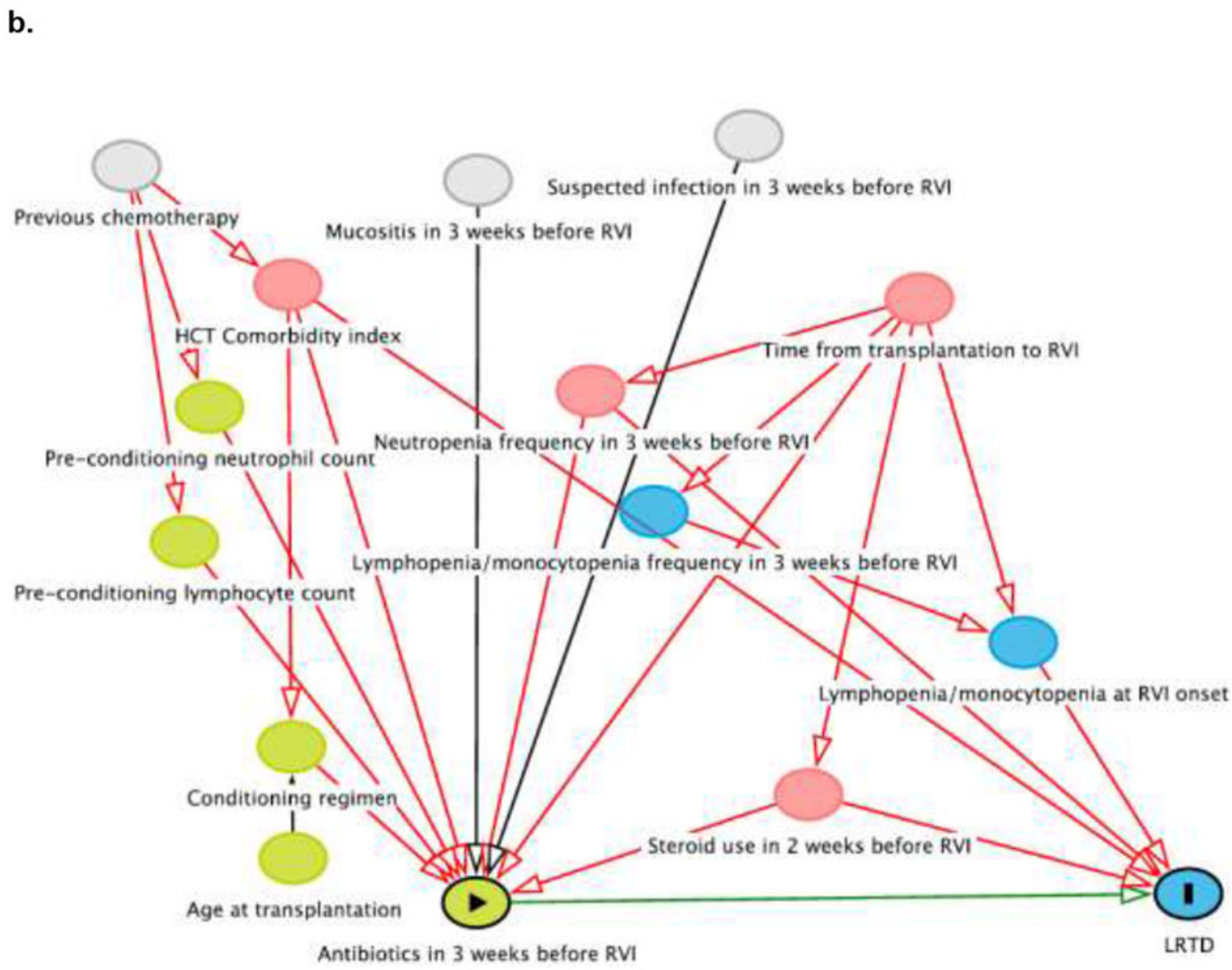


Figure 3a-3b. Directed acyclic graphs describing two hypothetical models for the relationship between our main exposure, antibiotic use in the three weeks prior to one day before viral onset, and our outcome, proven/probable/possible lower respiratory tract disease.

Legend: Green variables represent ancestors of the exposure, blue variables represent ancestors of the outcome, and red variables represent ancestors of both exposure and outcome. Grey variables indicate unmeasured variables for this study. Green arrow shows the causal path that needs to be estimated while red arrows show biasing paths that need to be blocked in order to estimate the causal association.

a) Under this framework, minimal sufficient sets of adjustment variables required to estimate this association without bias include either: 1) lymphopenia at viral onset or monocytopenia at viral onset, frequency of neutropenia in the three weeks before viral onset, and steroid use in the two weeks before viral onset, or 2) time from transplantation to viral onset, frequency of neutropenia in the three weeks before viral onset, and steroid use in the two weeks before viral onset. (b) In this model, minimal sufficient sets of adjustment variables required to estimate this association without bias include either: 1) lymphopenia at viral onset or monocytopenia at viral onset, frequency of neutropenia in the three weeks before viral onset, steroid use in the two weeks before viral onset, and HCT comorbidity index or 2) time from

transplantation to viral onset, frequency of neutropenia in the three weeks before viral onset, steroid use in the two weeks before viral onset, and HCT comorbidity index.

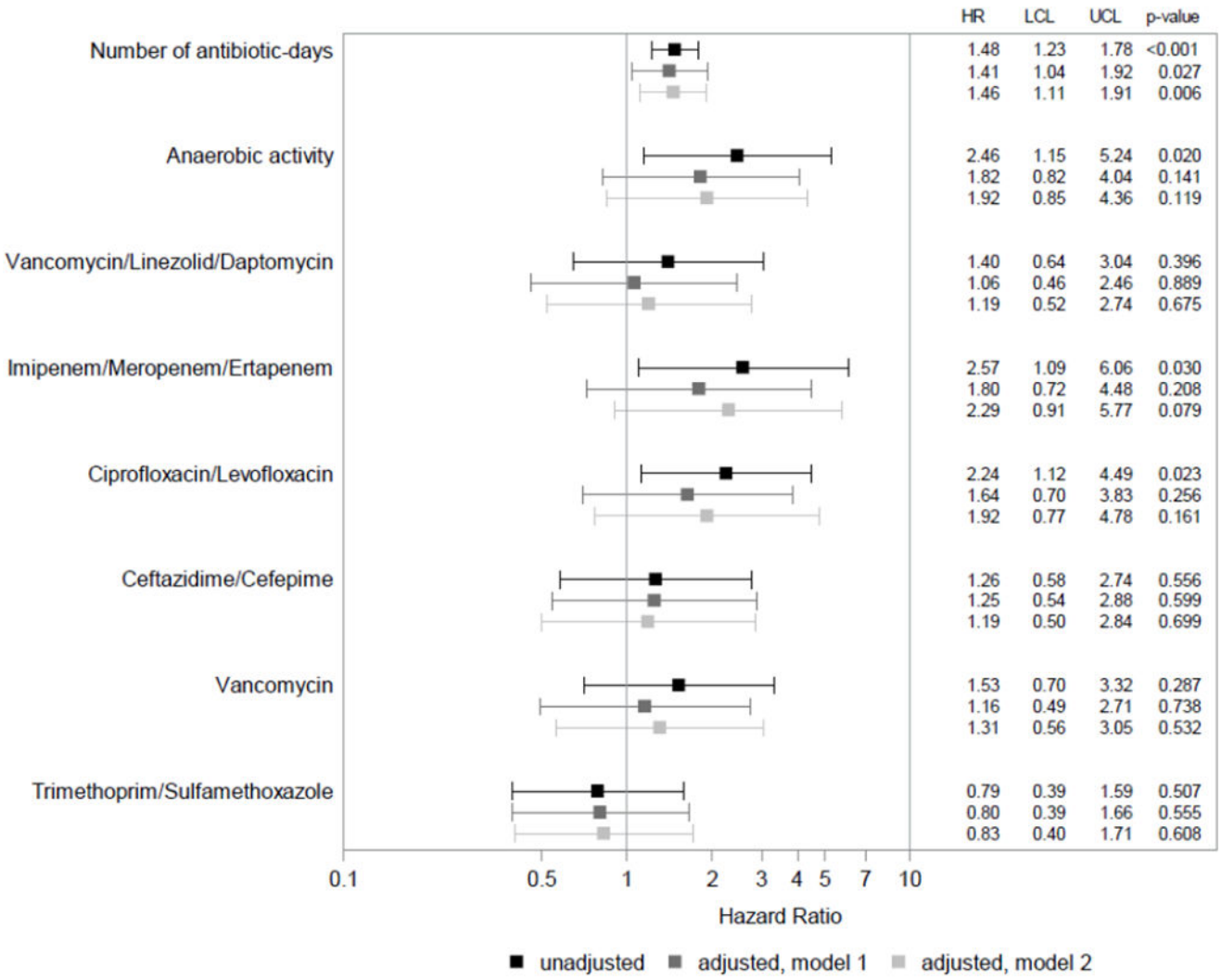


Figure 4. Model estimates for unadjusted and adjusted associations of antibiotic exposures in the three weeks prior to one day before viral onset with progression to proven/probable/possible lower respiratory tract disease

Legend: Filled squares represent hazard ratios (HR) and bars represent 95% confidence intervals (LCL = lower confidence limit; UCL = upper confidence limit). Estimates shown in black represent the unadjusted estimates, those in dark grey represent adjusted estimates from multivariable model 1 (adjusting for percentage of days with neutropenia in the three weeks before viral onset, lymphopenia at viral onset, and steroid use in the two weeks before viral onset), and those in light grey adjusted estimates from multivariable model 2 (adjusting for percentage of days with neutropenia in the three weeks before viral onset, monocytopenia at viral onset, and steroid use in the two weeks before viral onset). The HR for number of antibiotic-days represents the effect per 7-day increase; all other estimates represent any versus no use of the specified antibiotics. Antibiotics with anaerobic activity include meropenem, imipenem, ertapenem, piperacillin/tazobactam, ampicillin/sulbactam, amoxicillin/clavulanate, metronidazole, moxifloxacin and clindamycin.

Table 1.

Baseline Characteristics of Allogeneic Hematopoietic Cell Transplant Recipients with Upper Respiratory Tract Disease

Baseline Characteristic	Respiratory syncytial virus	Parainfluenza virus	Human metapneumovirus	All
Number of patients, n	23	51	16	90
Cohort, n (%)				
Cohort 1[a]	22 (96%)	45 (88%)	10 (63%)	77 (86%)
Cohort 2[b]	1 (4%)	6 (12%)	6 (38%)	13 (14%)
Male, n (%)	12 (52%)	30 (59%)	9 (56%)	51 (57%)
Age in years[c], median (range)	44 (3 - 63)	52 (6 - 77)	48 (17 - 59)	49 (3 - 77)
Adults, n (%)	21 (91%)	48 (94%)	15 (94%)	84 (93%)
Days from transplantation to viral onset, median (range)	45 (5 - 90)	55 (5 - 97)	46 (3 - 100)	51 (3 - 100)
Days from transplantation to viral onset, n (%)				
≤30 days	7 (30%)	14 (27%)	6 (38%)	27 (30%)
>30 days	16 (70%)	37 (73%)	10 (63%)	63 (70%)
Conditioning regimen, n (%)				
Myeloablative	19 (83%)	40 (78%)	9 (56%)	68 (76%)
Non-myeloablative	4 (17%)	11 (22%)	7 (44%)	22 (24%)
Graft source, n (%)				
Bone marrow	7 (30%)	5 (10%)	4 (25%)	16 (18%)
Cord blood	4 (17%)	6 (12%)	1 (6%)	11 (12%)
Peripheral blood stem cell	12 (52%)	40 (78%)	11 (69%)	63 (70%)
Hematopoietic cell transplantation comorbidity index[c], n (%)				
0	3 (13%)	3 (6%)	1 (6%)	7 (8%)
1-2	7 (30%)	19 (37%)	5 (31%)	31 (34%)
≥3	13 (57%)	29 (57%)	10 (63%)	52 (58%)
Lymphocyte count/mm^3, median (range)				
From 21 to 8 days before transplantation[d]	790 (100 - 2840)	610 (0 - 2090)	725 (130 - 2100)	685 (0 - 2840)
Lymphopenia, < 100/mm^3, n (%)				
At viral onset[e], n (%)	2 (9%)	6 (12%)	4 (25%)	12 (13%)
From 21 to 1 day before viral onset[f], median (range)	0.05 (0.00 - 1.00)	0.00 (0.00 - 1.00)	0.27 (0.00 - 0.90)	0.00 (0.00 - 1.00)
Neutrophil count/mm^3, median (range)				
From 21 to 8 days before transplantation[d]	1410 (50 - 4500)	1230 (0 - 9080)	1315 (30 - 4220)	1275 (0 - 9080)

Baseline Characteristic	Respiratory syncytial virus	Parainfluenza virus	Human metapneumovirus	All
Neutropenia, < 500/mm^3, n (%)				
At viral onset[e], n (%)	2 (9%)	11 (22%)	4 (25%)	17 (19%)
From 21 to 1 day before viral onset[g], median (range)	0.07 (0.00 - 1.00)	0.00 (0.00 - 1.00)	0.09 (0.00 - 1.00)	0.00 (0.00 - 1.00)
Monocyte count/mm^3, median (range)				
From 21 to 8 days before transplantation[d]	170 (0 - 670)	180 (0 - 1150)	165 (0 - 940)	175 (0 - 1150)
Monocytopenia, < 100/mm^3, n (%)				
At viral onset[e], n (%)	2 (9%)	10 (20%)	3 (19%)	15 (17%)
From 21 to 1 day before viral onset[h], median (range)	0.00 (0.00 - 0.85)	0.11 (0.00 - 1.00)	0.13 (0.00 - 1.00)	0.08 (0.00 - 1.00)
Highest daily dose of steroids in two weeks before viral onset, n (%)				
None	9 (39%)	25 (49%)	10 (63%)	44 (49%)
>0 to <1 (mg/kg)	10 (43%)	14 (27%)	5 (31%)	29 (32%)
1 to <2 (mg/kg)	4 (17%)	11 (22%)	0 (0%)	15 (17%)
≥2 (mg/kg)	0 (0%)	1 (2%)	1 (6%)	2 (2%)
Positive blood culture from 28 to 1 day before viral onset, n (%)[i]	3 (13%)	5 (10%)	2 (13%)	10 (11%)

[a] Clinical cohort, patients tested when symptomatic.

[b] Surveillance cohort, patients tested weekly regardless of symptoms. Only patients with respiratory symptoms at viral onset were selected.

[c] At time of transplantation.

[d] Using the minimum value per patient in time window.

[e] Using nearest value within two weeks before viral onset.

[f] Proportion of days per patient with lymphocyte count <100/mm^3.

[g] Proportion of days per patient with neutrophil count <500/mm^3.

[h] Proportion of days per patient with monocyte count <100/mm^3.

[i] Any bacteria or fungi growing from blood culture from 28 to 1 days before viral onset except for contamination.