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Detection of Human Cytomegalovirus in Bronchoalveolar Lavage Fluid of Lung Transplant Recipients Reflects Local Virus Replication and Not Contamination from the Throat[▽]

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Whether bronchoalveolar lavage (BAL) fluid may be contaminated with oropharyngeal cytomegalovirus (CMV) has never been investigated. In an analysis of CMV DNA loads in 76 simultaneously obtained BAL fluid and throat wash samples from lung transplant recipients, we show that such contamination is unlikely and that detection of CMV DNA in BAL fluid reflects virus replication in the lung.

Human cytomegalovirus (CMV) can cause CMV syndrome or end-organ disease in lung transplant recipients (LTRs) (11), as well as indirect effects on the allograft, such as bronchiolitis obliterans (9). Since virus replication in the lung is not always associated with viremia, detection of virus directly from the lung compartment is of utmost importance (10). Therefore, CMV detection from bronchoalveolar lavage (BAL) fluid samples is routinely performed during the follow-up of LTRs (8).

BAL fluid is routinely collected during bronchoscopy. Although this procedure does not necessarily lead to a fluid carryover from the throat to the lower respiratory tract, it could be possible that contamination from the pharynx to the lung occurs, thus leading to false-positive results by microbiological diagnostics from BAL fluid (2). This is especially important regarding CMV, since CMV replicates in cells of the host's pharynx during primary infection as well as during reactivation (6). While local CMV reactivation in the pharynx is usually clinically insignificant, detection of virus in the lung of an LTR is always considered a potentially serious diagnosis. Therefore, the aim of the present study was to clarify the relevance of such contamination during bronchoscopy for CMV diagnosis from BAL fluid.

Between December 2006 and October 2009, 76 parallel throat wash and BAL fluid samples were prospectively obtained from 63 LTRs, 38 (60.3%) male and 25 (39.7%) female patients, at the Medical University of Vienna. Nine patients were sampled twice, and two were sampled three times during the study period. Reasons for bronchoscopy were routine follow-up or clinical symptoms requiring BAL for diagnosis. On the day that bronchoscopy was performed, throat wash samples were collected first, followed by bronchoscopy.

The median time between transplantation and sampling was 372 days (range, 99 to 6,420 days). The underlying diseases requiring lung transplantation were chronic obstructive pulmonary disease (COPD) (46.0%), lung fibrosis (22.2%), cystic fibrosis (11.1%), and other causes (21.7%). Forty-seven patients (74.6%) had received a double lung transplant, 14 (22.2%) a single lung transplant, and two patients a combined heart-lung transplant (3.2%). The CMV donor (D) and recipient (R) serostatuses were as follows: 30.6% D+ R+, 29.0% D− R+, 16.1% D+ R−, 12.9% D− R−, and 11.2% unknown. All LTRs were on standard triple immunosuppression and did not receive (val)ganciclovir at the time of sampling. The ethics committee of the Medical University of Vienna approved the study.

Throat wash samples were obtained from the patients by having them gargle with 5 ml of sterile 0.9% saline solution for 10 s. BAL fluid was collected from the lung by instilling and retrieving 100 ml of 0.9% saline solution during bronchoscopy. Both sample types from the individual patients were tested for CMV DNA load in one test run, using a CMV Monitor test kit with a Cobas Amplicor analyzer (both from Roche Molecular Systems, Branchburg, NJ). Descriptive statistics, Fisher's exact test, and the Wilcoxon signed-rank test were done using SPSS 15.0.

First, we assessed the CMV DNA detection rate for the 76 throat wash and BAL fluid samples. In 49 (64.5%) of the sample pairs, the BAL fluid and throat wash samples were concomitantly negative for CMV DNA. In 11 (14.5%) of the sample pairs, CMV DNA was detected only in the BAL fluid (median, 3.72 $\log_{10}$ copies/ml), and in four cases (5.3%), CMV DNA was detected only in the throat wash samples (median, 2.21 $\log_{10}$ copies/ml), excluding contamination from the pharynx to BAL fluid in these cases. In the remaining 12 sample pairs (15.8%), CMV DNA was found in both BAL fluid and throat wash samples (Table 1).

To address the question of whether the positive BAL result for these 12 sample pairs could be due to contamination from the pharynx, we compared the CMV DNA loads detected in these samples. As shown in Table 1, the BAL fluid CMV DNA load was significantly higher than that of the corresponding throat wash sample in 9 of the 12 sample pairs ($P = 0.008$, Wilcoxon signed-rank test), thus excluding contamination. In the other three concomitantly positive sample pairs, the CMV DNA load in throat wash samples exceeded that detected in BAL fluid (Table 1), but in only one case was the difference

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TABLE 1. CMV DNA loads detected for 27 patients with positive BAL fluid and/or throat wash samples[a]

Result (log_{10} copies/ml) for sample type indicated		
Patient	BAL fluid	Throat wash
1	ND	2.00
2	ND	2.20
3	ND	2.21
4	ND	2.64
5	2.00	ND
6	2.34	ND
7	2.45	ND
8	3.11	ND
9	3.28	ND
10	3.72	ND
11	3.78	ND
12	3.90	ND
13	3.93	ND
14	4.27	ND
15	5.09	ND
16	2.62	2.03
17	3.05	2.06
18	3.53	2.00
19	3.54	3.14
20	3.68	2.00
21	4.14	2.66
22	4.52	2.31
23	4.58	3.64
24	5.20	4.89
25	2.76	3.32
26	3.47	4.08
27	3.01	4.79

[a] ND, CMV DNA not detected.

more than one log. When the CMV DNA loads detected for the entire set of 76 sample pairs were compared, the Wilcoxon signed-rank test showed that the CMV DNA loads in the BAL fluid were significantly higher than in the throat wash samples ($P = 0.009$).

To our knowledge, this is the first study to assess the relationship between CMV DNA loads detected in simultaneously obtained BAL fluid and throat wash samples from LTRs. Contamination of BAL fluid with resident or environmental microorganisms and appropriate measures to address this during microbiological diagnostics have been previously described (3). However, the possible risk of contamination of BAL fluid with CMV has been unclear so far. Earlier studies showed that shedding of CMV DNA in saliva occurs depending on the patient's clinical situation. Virus was detected in the saliva of 1.7 and 6.7% of healthy adults as opposed to 15.5 and 29.5% of HIV-positive patients in two previous studies (4, 5) and in up to 45.2% of patients after stem cell transplantation in another study (1). Consistent with these data, CMV DNA was detected in throat wash samples in 21.1% of the LTRs investigated in the present study, indicating a potential contamination risk.

However, from the present findings, it appears that CMV contamination of BAL fluid is a very rare event, if it happens at all. Contamination could be clearly excluded in the 81.3% of BAL fluid samples that were collected at a time point when the corresponding throat wash samples were positive. Together with the observation that DNA loads in BAL fluid were overall significantly higher than in throat wash samples, this argues strongly against contamination of the lung compartment with CMV during bronchoscopy. Our results are also supported by virus isolation data from HIV patients showing that CMV recovery rates from BAL fluid and induced sputum samples were, overall, higher than from saliva samples (7). Still, in three cases in our study, the CMV DNA load in the throat wash sample exceeded that in BAL fluid. However, in at least two cases, the differences were smaller than 1.0 log_{10} copies/ml; therefore, taking the BAL fluid dilution factor into consideration, the positive BAL results in these cases are unlikely to be due to contamination.

In conclusion, contamination of BAL fluid samples with CMV DNA from the oropharynx seems to be highly unlikely in LTRs, and the CMV DNA load data obtained from 100-ml samples of BAL fluid thus reliably reflect CMV replication in the lung.

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